UNIVERSITY OF TORONTO
FACULTY OF APPLIED SCIENCE & ENGINEERING

Report on

BioZone

Centre for Applied Bioscience and Bioengineering

2017

Report on BioZone
July 2017

Reporting Period September 2015 - August 2016

An electronic copy of this report is available through www.biozone.utoronto.ca.

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Director's Message

Welcome to BioZone, a Centre for Applied Bioscience and Bioengineering at the University of Toronto.

Elizabeth Edwards
Professor and Director, BioZone

BioZone is a research and training centre that brings together engineers, biologists, environmental and health scientists at the University of Toronto. BioZone has grass roots beginnings – folks coming together because it made sense to do so – to share a variety of often expensive equipment, and to share knowhow and support staff. It has become a place where students can access all kinds of state-of-the art equipment, hear different perspectives, learn to listen and share ideas, make mistakes, and witness the value of open and honest teamwork. Our goal is to create a place where new thinking and skills can be learned and vetted in a supportive dynamic environment.

A lot has happened since our last *Report on BioZone* in 2013. In 2013, the >$4M CFI/MRI infrastructure project that enabled the construction of BioZone space was completed, large research grants funded by Genome Canada and the Province of Ontario were drawing to a close, and the next wave of collaborative initiatives were being dreamt up and drafted. While we are certainly remiss to not have had a report since 2013, the truth is that these reports take a tremendous effort to put together, and we simply did not have the person-power to adequately collect the data and pull it together until now. Fortunately, we were able to secure renewed major funding, starting with the $5M NSERC Industrial Biocatalysis Network in 2014, followed by two Faculty of Applied Science and Engineering (FASE) Dean's Strategic Funds (DSF) to support an Executive Director position and our Mass Spectrometry Facility. After considerable proposal writing and defending in 2015-16, several large grants led by BioZone PIs were awarded in 2016, finally ensuring stable funding for the next 4 to 5 years. These new projects, highlighted in this document, include tackling environmental liabilities in the mining industry, finding new uses for lignocellulosic polymers or new microbes for aquifer remediation, growing algal biofilms for wastewater treatment and carbon dioxide sequestration, and producing renewable biochemicals. These projects land smack in the nexus of Environment, Energy and Water to create a healthier planet for all. Several new BioZone projects have a direct health connection, focusing on nutrition and food fortification and tissue engineering.

It is exciting to have secured renewed research funding, but even more exciting to be surrounded by wonderful colleagues, students, post docs, research associates, technicians, and staff from all around the world. I am particularly thrilled with the many new large team grants that leverage BioZone's capacity. I'm also thrilled to have such a diversity of people and experiences in BioZone. More and more, we are attracting short-term visiting students and professors from around the globe.

With the support of many individuals, but particularly from Grant Allen, the Chair of Chemical Engineering and Applied Chemistry, and from our Dean, Cristina Amon, we were able to recruit Sean Caffrey to BioZone as our Executive Director. Please read a little about Sean in the pages that follow. He has already taken a leading role in the development and management of BioZone's large projects, as well as helping define roles and responsibilities for the day-to-day operation of BioZone. He has empowered students by helping them form the BioZone Student Council, and most of all, he can engage action from all of us with a timely reminder (or several), a smile and his hard work.

Elizabeth A. Edwards

Elizabeth Edwards, Director

Executive Director's Message

BioZone's Four Pillars of Sustainability

At BioZone, we spend a great deal of time thinking about sustainability. This includes the traditional three pillars of sustainability: economic, social, and environmental, plus ensuring that BioZone itself is sustainable.

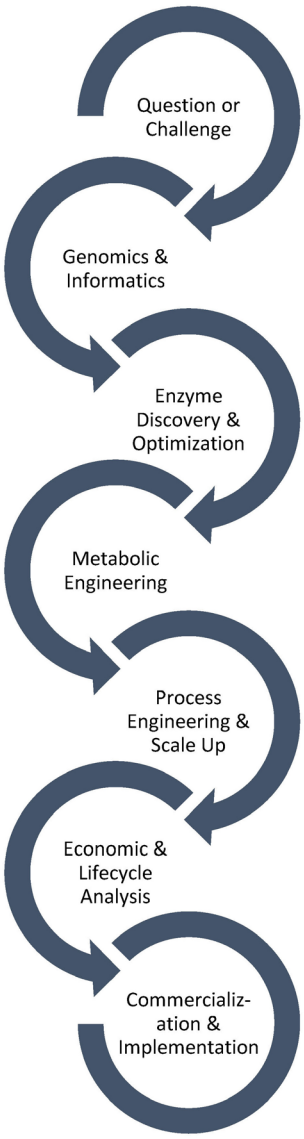
Most, if not all, of the research conducted at BioZone has a direct impact on environmental, economic, and social sustainability. BioZone researchers are discovering biological solutions to industrial-scale problems, typically involving microorganisms or enzymes. This is exemplified by the new large-scale grants received by BioZone over this past year. They involve designing greener industrial waste treatment processes for the forestry and mining industry, reducing the environmental footprint of manufacturing processes by replacing petrochemical feedstocks with renewable biomass derived from agricultural and forestry waste, and the use of microbial communities to remediate groundwater contaminated by industrial pollution. We believe that environmental sustainability and economic sustainability are not mutually exclusive. By helping industry to become sustainable and developing products desired by environmentally-conscious consumers, BioZone research can help grow the Canadian economy and support job growth. This is particularly true of biotechnologies that help provide new markets for Canada's agricultural and forestry sectors and support supply chain jobs in rural communities. In addition to the social benefits derived from living in a clean and healthy environment, BioZone's research on low-cost micronutrient addition to foods (e.g., salt fortified with iron) is helping alleviate the serious health issues associated with anemia for millions of people in developing countries.

Although a much smaller-scale problem than the fate of the planet, the sustainability of BioZone is something we are mindful of. Clearly, the funding obtained by securing large-scale grants is critical for financing the research that drives BioZone and the equipment that facilitates the research. BioZone's continued funding success will rely on our ability to demonstrate to funders and donors that the research we undertake has a measurable impact. Publication numbers and invention disclosures are important metrics for impact, however funders are increasingly interested in supporting research that directly impacts society by being implemented as industrial practice or developed as a commercial product. Although BioZone has a strong track record of research translation, as measured by patents, company creation, and deployment of our research by industry, we are always working to improve our impact. Thus far, we have achieved our success by deeply integrating industry partners capable of commercializing biotechnology into our large research projects. Going forward, we hope to expand our research translation efforts by promoting BioZone as a hub where industry gathers to solve their urgent problems by accessing leading-edge equipment and expertise. A step in creating this industry hub in BioZone is establishing relevant fee-for-service facilities open to the private sector. BioZone already has the Mass Spectrometry Facility that is attracting industrial users, and we hope to expand the services we offer to biomanufacturing (fermentation) along with protein production and purification. The goal isn't to make these facilities self-sufficient but to make connections to new industry partners with relevant problems to solve and the capacity to translate impactful BioZone research.

The past year was marked by many successes described in this report including new grants, numerous publications and awards, and the deployment of BioZone technologies through existing and new companies. With the initiation of exciting new research projects, I am sure the next year will be just as successful.

Sean Caffrey, Executive Director, BioZone

What is BioZone?

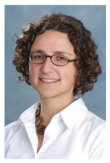


D. Grant Allen
Environmental bioprocess engineering

Krishna Mahadevan
Metabolic systems engineering

Alexei Savchenko
Enzyme crystallography

Levente Diosady
Food engineering



Emma Master
Enzymes for plant bioproducts

Alexander Yakunin
Enzyme genomics

Elizabeth Edwards
Bioremediation and anaerobic digestion

Alison McGuigan
Tissue engineering

Bradley Saville
Bioprocess technology & economic analysis

BioZone is a centre for applied bioscience and bioengineering research at the University of Toronto with a track record of developing sustainable technologies that reduce resource-use and help to clean up the environment. We are built upon the principles of collaboration, teamwork, and research excellence. BioZone is unique in that it is structured to provide extensive shared multidisciplinary laboratory space and research equipment that promotes cooperative research among a team of diverse professors, students, and staff of more than 130 people. In addition to a state-of-the-art mass spectrometry facility, BioZone includes extensive shared lab facilities that house an impressive array of sophisticated instruments, and collaborative student workspaces that accommodate visiting and local students.

BioZone researchers bring the tremendous advances in fundamental genome science to real-world industrial problems in manufacturing, forestry, mining, and energy production. BioZone also maintains strong links to health and biomedical research where there are many overlaps in methodology and approach, as well as protein and enzyme discovery, and therefore much to share. BioZone solutions have improved the sustainability of the world's biggest industries, helping them to achieve and exceed corporate social responsibility goals. With the resident expertise to address relevant technical, economic, and public policy constraints, BioZone's researchers have the ability and the passion to transform ideas from the laboratory to commercial application with the assistance of a large number of domestic and international collaborators. BioZone expertise includes: bioinformatics, genomics, metagenomics, proteomics, metabolomics, enzymology, structural biology, microbiology, cellular biology, chemistry, biophysics, catalysis, tissue culture, process engineering, mathematical modeling, and computer simulation.

BioZone connects engineering know-how with the power of genomics to address urgent challenges in energy, the environment and health.

Vision & Mission

BioZone's vision is to be a multidisciplinary and internationally recognized centre for bioengineering research, technology transfer, outreach and training focused on urgent societal needs in sustainable energy, the environment and human health.

A particular strength will be our ability to effectively translate the most appropriate and up-to-date molecular and cellular discoveries and tools into industrial practice.

BioZone's mission is to:

- attract the best and brightest minds from a diverse range of fields, including chemical and process engineering, microbiology, genomics, biochemistry, medicine, computer science, economics and public policy
- create a focal point for collaborative applied and environmental bioengineering research leading to the development of innovative new technologies that address urgent challenges and foster the long-term sustainability of our planet and its inhabitants
- provide state-of-the-art facilities and exciting opportunities for research
- provide students with the knowledge and ability to debate public policies and influence political decisions that affect the environment and health, based on sound scientific principles
- foster innovation, creativity, and imagination
- encourage leadership and excellence, humility and collegiality
- have a lasting and positive impact on our environment and society
- have fun

Executive Summary

This past year has been a year of transition. In 2014-2015, several of BioZone's large collaborative research grants came to an end. Consequently, a great deal of effort over the past year (September 2015 - August 2016) was spent applying to new large-scale research grants that included multiple BioZone researchers and collaborators beyond the University of Toronto. These efforts were aided by the addition of an Executive Director to BioZone who arrived in September 2015. Thankfully, we successfully secured two Genome Canada Grants, two Ontario Research Fund - Research Excellence grants and renewed a large National Institutes of Health grant. Although these research projects span multiple sectors (including forestry, chemicals, mining, and health), they primarily focus on using microbes and enzymes to make industrial processes less environmentally impactful and reduce greenhouse gas emissions. This new funding will allow BioZone to continue conducting important research and training.

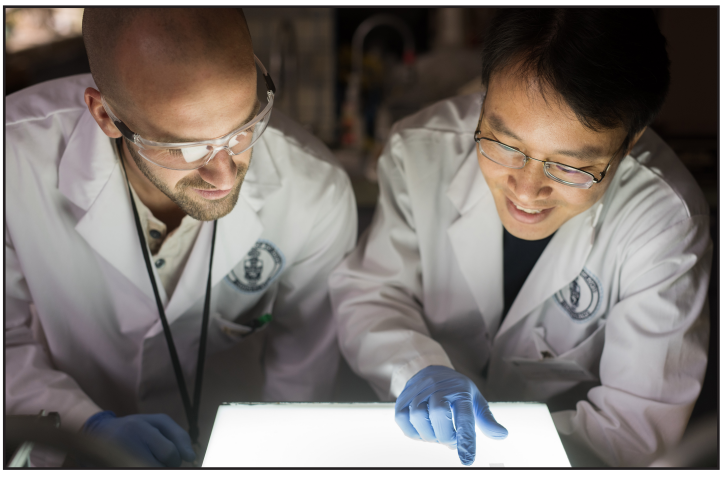
Continuing with the theme of transitions, 2016 saw the beginning of laboratory renovations to BioZone's biomanufacturing lab and adjacent office spaces. This effort was funded by the federal government's Strategic Initiative Program. BioZone also expanded to Calgary with the move of Dr. Alexei Savchenko to a tenured position at the University of Calgary with a portion of his lab.

Examples of research impact from this reporting period includes the launch of a sustainable chemicals company that will reduce the world's need for petrochemical feedstocks, the development of anaerobic digestion technology that will be deployed at scale to reduce municipal waste and capture greenhouse gases, the remediation of heavily-contaminated sites, and the delivery of salt fortified with iron to millions of individuals in India to fight anemia. In addition to these applied impacts, BioZone researchers published 48 publications in many high impact journals during this reporting year (2015-2016).

BioZone faculty and staff were recipients of several prestigious awards. Professor Diosady was named a fellow of the Royal Society of Canada, Professor Edwards was awarded the Killam Prize, and Professor McGuigan was awarded a Hart professorship, to name a few.

BioZone is continuing to expand beyond its boundaries. In addition to providing fee-for-service mass spectrometry services for our faculty and commercial users across the greater Toronto area, we are expanding to offering fermentation and protein cloning, production and crystallization services. We are pleased to have many collaborators and associate members of BioZone, who use the facilities intermittently including, for example, Professors Vlad Papangelakis, Elodie Passeport, Arthur Chan, Barbara Sherwood Lollar and Brent Sleep (see "*BioZone Members*" on page 13).

Overall, during the 2015-2016 reporting year, BioZone received over $4.2M in cash funding, as well as valuable and generous in-kind contributions from many industrial partners. A significant proportion ($1.5M) was secured from international organizations. BioZone was home to 104 students, PDFs, research assistants, technicians and staff. These and other metrics are provided in more detail in the following pages. We look forward to a coming year full of promise!



Executive Committee Report

BioZone continues to achieve internationally-recognized research excellence through a culture of collaboration and sharing. Our recent successes in large funding competitions from Genome Canada and the Ontario Research Fund are a testament to the high quality and impact of the research conducted by BioZone researchers. This funding allows BioZone to provide an outstanding learning and research environment for technological innovation in energy, environment, and health.

This report highlights our achievements during the September 2015 - August 2016 reporting period, unless otherwise indicated.

BioZone Research Impact

Chemicals From Renewable Feedstocks – *Ardra Bio*

Through the NSERC Industrial Biocatalysis Network and other projects, BioZone researchers and students are identifying and optimizing enzymes and metabolic pathways that can transform renewable biomass into useful specialty chemicals and materials.

One such technology is being commercialized through a renewable chemicals startup called *Ardra Bio*. *Ardra Bio*'s core technology is based on designer biochemical pathways that convert renewable biomass into high-value specialty and fine chemicals. *Ardra Bio* is currently using the technology to produce natural 1,3-butanediol from renewable green feedstocks that will replace petroleum-based 1,3-butanediol in cosmetics and personal care products. Using designer pathways, *Ardra* has further developed a portfolio of high-value natural flavour and fragrance ingredients.

Ardra Bio was incubated in the prestigious San Francisco-based Indie Bio-Accelerator and is now housed in JLabs at the MaRS Centre in Toronto. *Ardra* has received significant customer traction through offers for collaboration and co-development agreements for multiple products in its portfolio.

Life Cycle and Technoeconomic Assessment – *Savant Technical Consulting*

BioZone researchers operate *Savant*, a research-driven consulting company that provides world-leading expertise in the fields of microbiology and biotechnology, process and bioprocess design, financial modeling, and life-cycle assessment (LCA) for the renewable energy, environmental remediation, biofuels, and waste management industries.

Micronutrient Food Supplements - *Tata Trusts / The Bill and Melinda Gates Foundation*

BioZone researchers have developed a cost system for adding iron to salt without modifying its taste or appearance. To help combat the extremely high prevalence of anemia in developing countries, the iron-fortified salt developed in BioZone is being distributed to more than 24 million people in India, curing over 1 million children.

Remediation of Contaminated Sites - *DuPont* and *SiREM*

Microbial-based technology developed at BioZone and commercialized by SiREM is being used to remediate sites around the world contaminated with toxic chlorinated compounds. We are currently starting field trials that will assess the ability of microbial communities to remediate sites contaminated with benzene and other toxic petroleum-based compounds.

Processing Solid Organic Waste – *Miller Waste Systems Inc.*

BioZone researchers have developed and are testing new anaerobic digester designs that reduce the quantity of solid waste sent to landfills, produce renewable energy, lower the amount of greenhouse gases released into the environment, and generate a compost by-product suitable for land application. The benefits are considerable; a reduction in organic waste to landfills by up to 80%, a reduction in greenhouse gas emissions of 1.25 tonnes of CO_2 for every tonne of waste processed, and the production of commercial quantities of electricity or renewable natural gas.

This reactor design will be tested at demonstration scale, and then full scale, at a new integrated waste management facility to be constructed in Ottawa by Taggart Miller, a joint venture of The Miller Group and The Taggart Group.

Innovations

Some of the recent innovations from BioZone projects and researchers include:

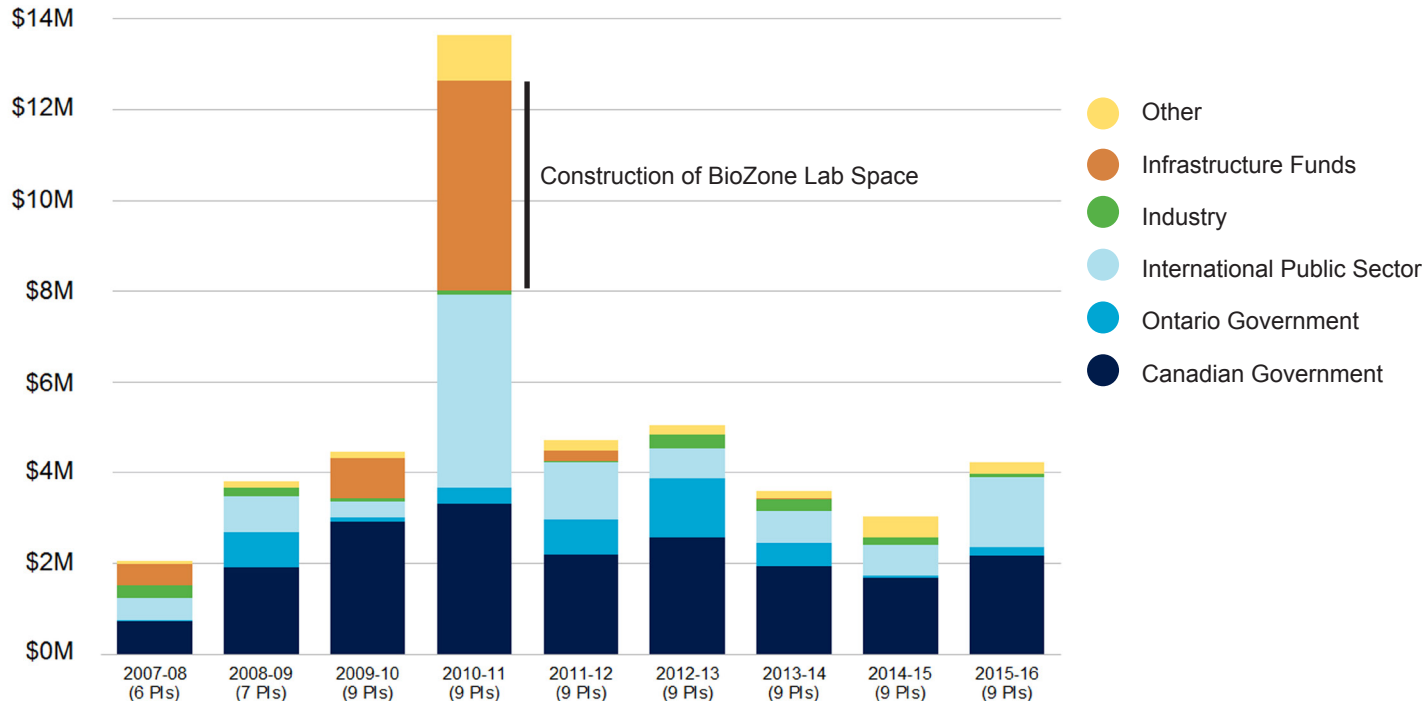
- Microbes engineered for 1,3-butanediol production, adipic acid, ethylene glycol, and other chemicals
- Novel enzymes for recycling of plastics made from polylactic acid
- A method to spatially and chemically resolve lignin from polysaccharides in woody samples
- Processes for fortifying salt with iron and iodine (double-fortified salt)
- Three-dimensional tissue modeling technology
- Benzene-degrading cultures for bioremediation
- Wave guides for enhanced microalgae production
- Novel anaerobic digester designs for municipal waste reduction

Research Funding

BioZone researchers worked on 112 funded research projects from September 2012 to August 2016, many of which involved collaborations between multiple research groups, domestic and international. Figure 1 shows the total research cash funding to the core BioZone principal investigators (PIs) since BioZone's inception. These totals do not include in-kind funding or project funds flowing to non-BioZone researchers from the awarded grants.

Cash funding for BioZone research in 2012-2016 came from a mix of domestic and international sources (Fig. 2). International research grants provided between 12-35% of total cash funding to BioZone from 2012-2016. The majority of funding for BioZone included funding from the federal government, particularly NSERC. This includes a seven-year Canada Research Chair in Anaerobic Biotechnology awarded to Dr. Elizabeth Edwards in 2014. Funding from the new Genome Canada and Ontario Research Fund grants did not start in this reporting period and are not reflected in the numbers below. You can read more about these grants on page 11.

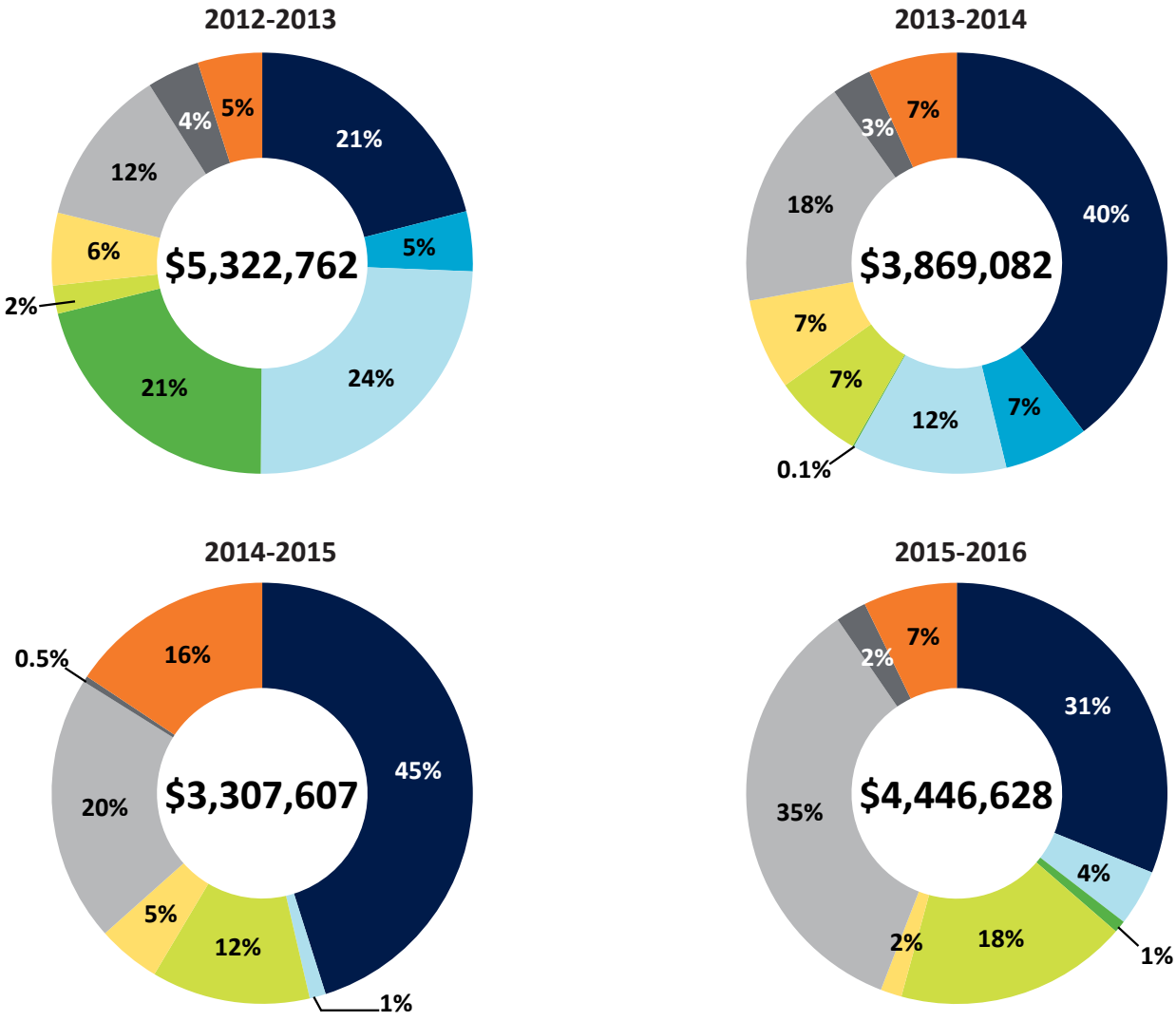
Figure 1 (and associated table below). BioZone cash funding received per year (September 1 to August 31). Includes operating, capital, and equipment grants, a Killam Fellowship, a Canada Research Chair, overhead, and amounts sub-awarded to other University of Toronto professors when a BioZone PI leads the project. Does not include in-kind contributions, amounts sub-awarded to other universities, or other leveraged funds.



	Canadian Government	Ontario Government	International Public Sector	Industry	Infrastructure	Other	Total
2007 / 2008	$737,813	$20,000	$486,799	$275,899	$480,179	$57,065	**$2,057,754**
2008 / 2009	$1,911,799	$784,218	$778,088	$190,568	$9,500	$135,512	**$3,809,685**
2009 / 2010	$2,925,232	$81,988	$354,655	$75,000	$887,828	$140,715	**$4,465,418**
2010 / 2011	$3,330,119	$334,065	$4,251,247	$93,155	$4,628,118	$1,009,180	**$13,645,884**
2011 / 2012	$2,198,925	$760,775	$1,267,228	$25,000	$234,748	$495,571	**$4,982,247**
2012 / 2013	$2,584,531	$1,303,452	$647,492	$296,204	$13,500	$477,584	**$5,322,763**
2013 / 2014	$1,950,982	$501,763	$695,648	$270,420	$13,496	$436,774	**$3,869,082**
2014 / 2015	$1,693,656	$41,300	$677,411	$160,993	$0	$734,246	**$3,307,607**
2015 / 2016	$2,167,201	$191,898	$1,536,707	$72,500	$0	$478,322	**$4,446,628**

Figure 2. Sources of cash funding to BioZone professors between September 1 to August 31 for the fiscal years between 2012 and 2016. Inclusions and exclusions are listed in the caption for Fig. 1.

Source	2012-13	2013-2014	2014-2015	2015-2016
NSERC	$1,119,289	$1,536,275	$1,492,606	$1,384,129
CFI	$244,618	$251,564	$0	$0
MEDI / MRI	$1,303,452	$461,763	$41,300	$191,898
Genome Canada	$1,119,123	$4,000	$0	$41,553
Other Canadian Government	$115,000	$267,349	$402,776	$794,456
Industry	$296,204	$270,420	$160,993	$72,500
International Public Sector	$647,492	$695,648	$677,411	$1,536,707
Nonprofit Organizations	$214,064	$118,544	$15,000	$105,000
UofT FASE & Chemical Engineering & Applied Chemistry	$263,520	$263,520	$517,520	$320,385
Total	**$5,322,762**	**$3,869,082**	**$3,307,607**	**$4,446,628**



Existing and New Major Research Projects

Major research initiatives in BioZone typically involve collaborations between multiple BioZone PIs, researchers at other institutions in Canada and abroad, and private sector partners. Thus, total project values for major research initiatives are often significantly higher than the cash awards as they include leveraged contributions from other institutions and private sector partners. Major awards held in September 2015 to August 2016 are listed below.

NSERC Strategic Network: Industrial Biocatalysis Network (2014-2019)

Website: *http://www.ibnet.ca/*

Member(s): Edwards (co-lead), Savchenko (co-lead) Mahadevan, Master, Yakunin and 4 other collaborators

Total project size: $5,915,000
Contributions:
NSERC: $5,000,000
Universities (Toronto, UBC, & Concordia): $420,000
Industry Partners (BP, CanSyn, DuPont, Elanco, Lallemand, Monaghan Biosciences, BP & Suncor): $495,000

Through the *Industrial Biocatalysis Network*, university researchers and industry partners are using the latest genomics tools to identify and characterize enzymes that can convert renewable resources, such as agricultural or forestry waste, into new materials and chemicals. The goal of the project is to use these enzymes to develop sustainable bioprocesses that reduce energy consumption and carbon emissions compared to traditional petrochemical processes.

Genome Canada Genomics Applied Partnership Program: Tackling Anaerobic Benzene Contamination with Anaerobic Microbes (2016-2019)

Website: *http://anaerobicbenzene.ca/*

Member(s): Edwards (lead)

Total project size: $997,397
Contributions:
Genome Canada: $317,422
Ontario Ministry of Research and Innovation: $317,422
SiREM: $217,540
Federated Cooperative Limited: $100,000
Mitacs: $45,000

This project is optimizing and scaling-up microbial cultures and monitoring tools that will drive *in situ* remediation of benzene and BTEX compounds. Outcomes include:
- significantly decreasing the time to clean up sites
- reducing the cost to meet regulatory requirements
- avoid disrupting on-going site activities
- reducing the costs of site monitoring

NIH/NIAID Contract: Center for Structural Genomics of Infectious Diseases (CSGID) (2012-2017)

Website: *http://www.csgid.org*

Member(s): Savchenko (lead)

Contributions:
National Institute of Allergy and Infectious Diseases, National Institutes of Health: $2,750,000

The *Center for Structural Genomics* (CSGID) is a multi-site collaborative initiative to determine the 3D crystal structures of bacterial proteins implicated in infectious disease. The goal of the project is to disseminate molecular information into the public domain (Protein Databank) to facilitate molecular biology research into the molecular mechanisms of infectious diseases and to accelerate targeted drug discovery efforts to treat such infections. The BioZone Protein Production and Crystallization Facility's role in the CSGID is to conduct high-throughput protein purification, crystallization, structure determination and interpretation/analysis. To date, the BioZone Protein Production and Crystallization Facility contributions to the CSGID include 2,300 cloned genes, 1,100 purified proteins, 405 protein crystals and 250 3D structures.

Saving Lives at Birth Consortium - Quadruple Fortification of Salt (2016-2018)

Website: *https://www.savinglivesatbirth.net/*

Member(s): Diosady (lead) and 1 other collaborator

Total Project Size: $331,710

Contributions:
United States Agency for International Development (US AID)
Norwegian Ministry of Foreign Affairs
The Bill & Melinda Gates Foundation
Grand Challenges Canada
The Department of International Development United Kingdom of Great Britain and Northern Ireland (DFID)
Korea International Cooperation Agency (KOICA)

This funding is administered by Grand Challenges Canada. Salt is universally consumed at a constant level independently of socio-economic status. We propose to fortify salt with iron, iodine, vitamin B12 and folic acid. Quadruple-fortified salt (QFS) will provide these at levels that will result in a substantial improvement in the micronutrient status of women and children, leading to large decreases in maternal, neonatal and infant mortality.

Upcoming Projects in BioZone

Exciting projects are continually on the horizon in BioZone. Here are two projects led by BioZone PIs that started following the reporting period of this report.

Genome Canada Large Scale Applied Research Project: SYNBIOMICS: Functional genomics and techno-economic models for advanced biopolymer synthesis (2017-2020)

Website: *http://www.synbiomics.ca/*

Member(s): Master (lead), Edwards, and 4 other collaborators

Total project size: $9,989,427
Contributions:
Genome Canada: $2,830,871
Genome Quebec: $791,438
Genome BC: $181,879
European Research Council: $2,298,124
Ontario Ministry of Research and Innovation: $479,318
Canadian Foundation for Innovation: $181,879
University of Toronto: $695,606
Queen's University: $60,000
Industry Partners (Canfor, DuPont, EcoSynthetix, IGPC, Innotech Millar Western, Tembec, West Fraser): $635,000

The *SYNBIOMICS* project is developing biocatalysts that upgrade the three wood fractions produced by pulp mills: cellulose, hemicellulose, and lignin.

By upgrading, rather than degrading, these renewable biopolymers, this project overcomes major challenges of biotechnologies developed to breakdown lignocellulose structures and builds on the unique qualities of Canada's forest resource. The optimized biocatalysts developed through the *SYNBIOMICS* project will upgrade renewable forest biomass to create high-value bio-based polymers for targeted applications, including resins, coatings, bioplastics, and adhesives for lightweight biocomposites.

SYNBIOMICS will provide the following environmental, social, and economic benefits:

- environmental benefits by facilitating healthy forestry practices and promoting the transition from petrochemical to lower carbon renewable feedstocks;
- social benefits by revitalizing the forestry sector by increasing the sustainable forest harvest and establishing SME-mill clusters that drive economic activity and job growth in rural communities; and,
- economic benefits by creating new forestry-derived products such as biopolymers for use in adhesives, dispersants, coatings, and bio-resins to meet global demand for green bio-based products.

Ontario Research Fund - Research Excellence: Elements of Bio-mining (2017-2021)

Website: www.biomining.ca

Member(s): Papangelakis (lead), Edwards (co-lead), Mahadevan, Yakunin, Saville, Savchenko, and 7 other collaborators

Total project size: $12,000,000
Major Contributions:
Ontario Ministry of Research and Innovation: $4,000,000
NSERC: $1,257,792
Genome BC: $204,975
University of Toronto: $1,283,812
Laurentian University: $292,073
University of British Columbia: $349,481
CSIRO: $363,614
INMARE: $500,000
Industry Partners (Barrick, CEMI, Denison Environmental, Glencore, Hatch, MetagenomBio, Imperial, Teck, Tetratech, & Vale): $2,274,094

Issue: When sulfide minerals are mined and processed for base metal recovery, iron sulfides are rejected as waste tailings. When exposed to air, water, and microbes, these sulfide-laden waste tailings are oxidized to sulfuric acid, thereby producing acid mine drainage. The cost to clean up acid mine drainage at Canadian mine sites is estimated to be in the range of $2-5 billion. The value of the nickel stored in existing tailings in Sudbury, Ontario is estimated to be $7 billion. Thus, there is financial and environmental incentive to improve mine waste management. Selenium (Se) is an analog of sulfur (S) and a minor but more toxic component of sulfide minerals. Metal and coal mines in Canada released ~20 tonnes of Se into water in 2012.

Solution: With the tremendous advances in molecular biology tools, sequencing technology, and the ensuing "omics" revolution, bioprocesses are no longer "black boxes." Acid mine drainage ecology has now been characterized and provides us with a broad understanding of the dominant microorganisms in these communities. This information provides us with a toolkit to engineer improved mining processes by manipulating individual microbes or entire communities. This project will develop microbial-driven processes to stabilize S and Se in their neutral elemental forms with simultaneous base metal recovery. Conventional approaches stabilize S and Se in their most reduced state (as sulfide or selenide). Our new approach has technical and economic advantages, and also challenges. While microbial processes contribute significantly to the oxidation and reduction of S and Se, arresting these reactions at elemental S and Se while liberating metals of commercial value requires a better understanding of rate-controlling reactions and the microbes involved in these conversion processes.

Ontario Research Fund - Research Excellence: Biochemicals from Cellulosic Biomass (BioCeB) (2017-2021)

Member(s): Mahadevan (co-lead), Yakunin, Savchenko, and 5 other collaborators

Total project size: $ 11,816,407
Major Contributions:
Ontario Ministry of Research and Innovation: $3,929,469
NSERC: $1,343,885
University of Toronto: $1,144,865
University of Ottawa: $437,499
Industry Partners (BioAmber and Bioindustrial Innovation Canada): $2,550,000

The *BioCeB* project will design bacterial and yeast strains capable of efficiently converting glucose derived from renewable agricultural and forestry feedstocks into value-added chemicals such as adipic acid for bio-nylon production. The goal of the *BioCeB* project is to develop microbial manufacturing systems that reduce society's reliance on petrochemicals, reduce the use of toxic compounds, and lower greenhouse gas emissions.

Industry Support

BioZone gratefully acknowledges support from the wide range of public, nonprofit, and private sector partners listed on page 61 and 62 of this report, whose contributions have included personnel time, expertise, samples, equipment and research funding.

BioZone Members

Full Members

D. Grant Allen

Environmental bioprocess engineering

Krishna Mahadevan

Metabolic systems engineering

Alexei Savchenko

Enzyme crystallography

Levente Diosady

Food engineering of food

Emma Master

Enzymes for plant bioproducts

Bradley Saville

Bioprocess technology & economic analysis

Elizabeth Edwards

Bioremediation and anaerobic digestion

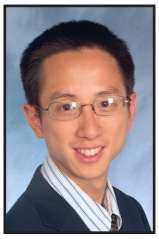
Alison McGuigan

Tissue engineering

Alexander Yakunin

Enzyme genomics

Associate Members



Arthur Chan

Atmospheric chemistry, air pollution, and health impacts

Elodie Passeport

Removal of contaminants from natural environments

Vlad Papangelakis

Aqueous and environmental process engineering

Barbara Sherwood Lollar

Deep subsurface geochemistry and microbial degradation

Brent Sleep

Biological and geochemical processes in soils

Recruiting Top Research Talent from Around the World

BioZone is home to a very diverse and gifted pool of students, researchers, staff and PIs from a wide range of disciplines. Since the centre's inception, we have attracted personnel and trainees from over 30 countries on 5 continents. Figure 3 below illustrates the geographical diversity of our current complement of BioZone researchers from twenty-five countries from all corners of the globe.

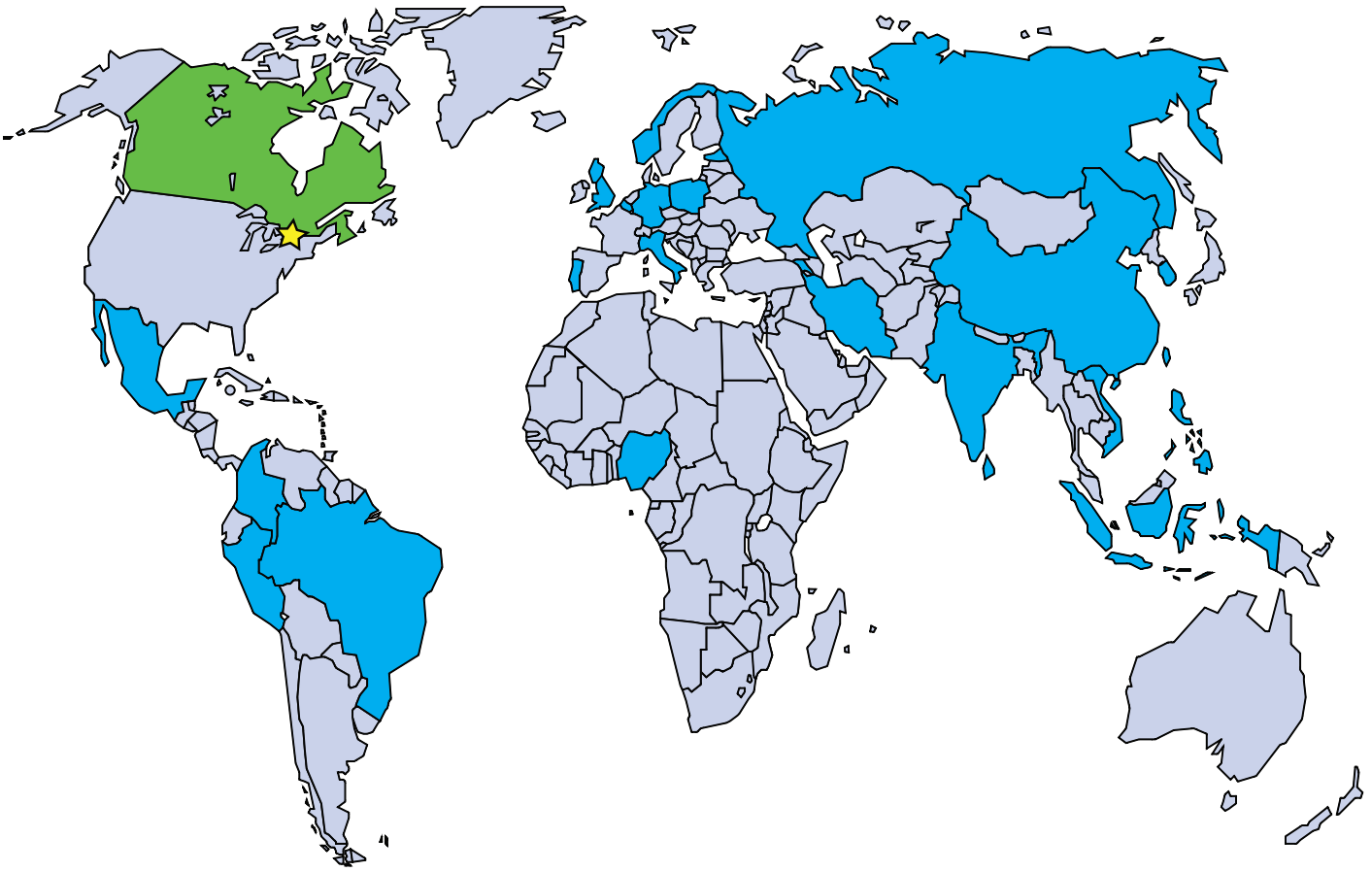


Figure 3. Countries of origin, highlighted in light blue, of BioZone researchers, graduate students and staff at the end of the reporting period. The location of the University of Toronto is denoted with a yellow star, and Canada is highlighted in green.

The interdisciplinary nature of BioZone's research community is depicted by the variety of departments represented by our personnel since its inception:

- Biochemistry
- Cell & Systems Biology
- Civil Engineering
- Chemical Engineering and Applied Chemistry
- Earth Sciences
- Ecology & Evolutionary Biology
- Geography
- Institute of Biomaterials & Biomedical Engineering
- Mechanical Engineering
- Medicine

At the end of this reporting period, BioZone was home to 104 graduate students, postdoctoral fellows, staff and PIs (Fig. 4). For the 2015-2016 reporting period alone, 20 undergraduate students gained valuable hands-on experience working in BioZone's laboratories including: six undergraduate theses, one co-op student and thirteen summer internships.

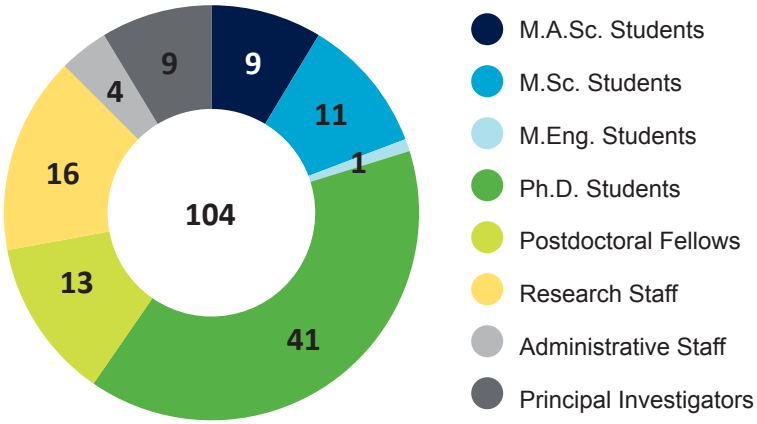


Figure 4. Headcount of BioZone personnel by category as of Aug. 31, 2016.

Dedication to Research

BioZone emphasizes education and mentorship with dozens of graduate and undergraduate students undertaking research projects. This is in great part due to the research support team in BioZone consisting of both laboratory technicians and research associates. The seventeen outstanding research staff (Fig. 5) in BioZone not only assist in the education of our next generation of researchers, but also ensure continuity of best practices and oversight of our long-term collaborative research programs.

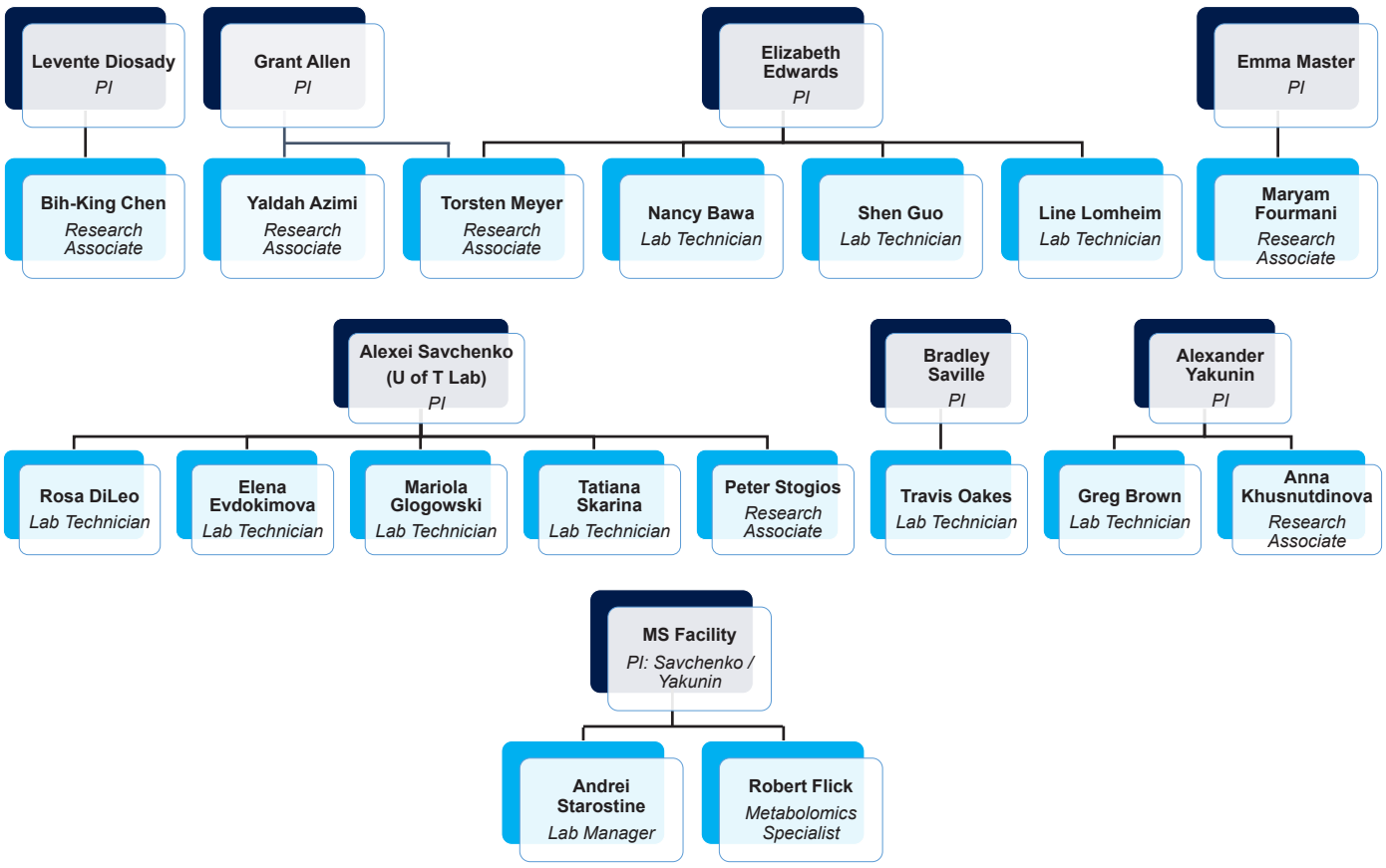


Figure 5. Distribution of research associates and laboratory technicians by principal investigator as of August 31, 2016.

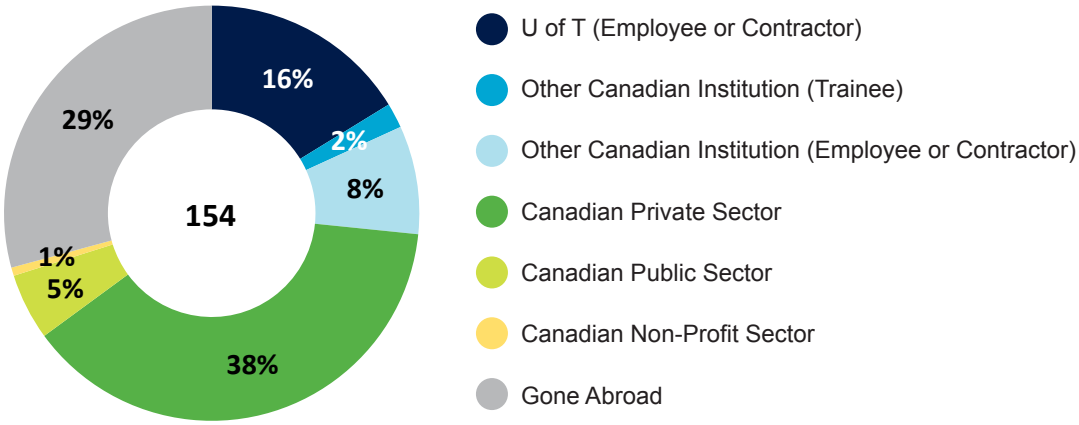


Figure 6. Breakdown of industry sectors reached by BioZone alumni, including former graduate students, postdoctoral fellows, research associates and technicians. Graph based on information for 151 BioZone alumni whose current positions are known.

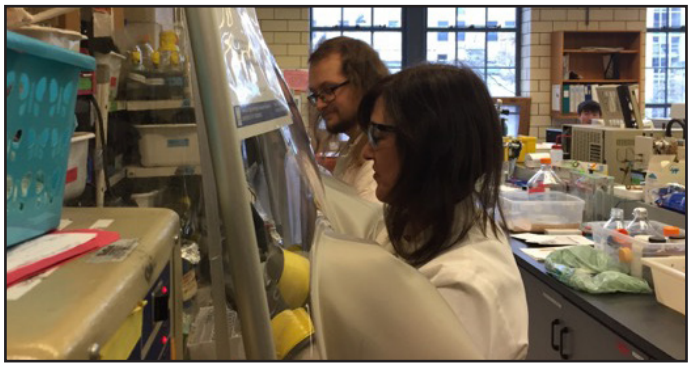
BioZone Alumni

Since the inception of BioZone in 2007, a total of 134 graduate degrees have been awarded to students in BioZone. This includes 68 M.A.Sc., 16 M.Eng., 1 M.H.Sc., 11 M.Sc. and 38 Ph.D. degrees. In addition, 39 postdoctoral fellows completed their fellowships, 73 undergraduate theses have been completed and well over 100 summer students have been mentored over this period. Alumni from BioZone have gone onto professional and academic positions in Canada and abroad. Fig. 6 shows the breakdown by sector for the BioZone graduates for which information is available.

Many of BioZone's graduates continue on to new and exciting endeavours in a variety of fields, including starting their own research labs, to breaking out and starting their own companies. For an update on just a few BioZone graduates, please refer to the *"Alumni Updates"* section on page 39.

BioZone alumni have branched out to all corners of the globe into a variety of career paths ranging from project management to outreach to academia. Some of the ventures our graduates have gone onto include:

- The American Chemical Society
- Apotex Inc.
- Bayer
- Gay Lea Foods Cooperative
- Golder Associates
- Government of Ontario
- Intrexon Corporation
- Johnson & Johnson
- Lanzatech
- Luminex
- NOVA Chemicals
- Novozymes
- Oak Ridge National Laboratory
- Pfizer Canada
- PwC
- Ramboll Environ
- Sanofi Pasteur
- ZymoResearch



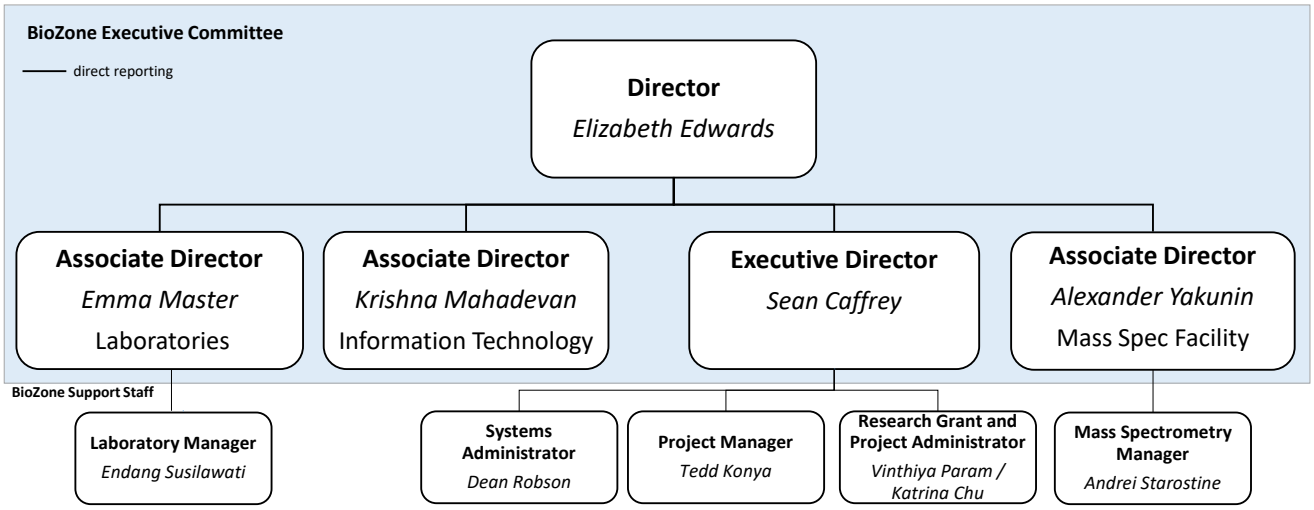


Figure 7. Overview of BioZone's leadership showing Executive Committee and Administrative and Management Staff.

BioZone's Director is appointed by the Dean of the University of Toronto's Faculty of Applied Science and Engineering, and reports administratively to the Dean. Elizabeth Edwards was appointed as BioZone's founding Director in January 2011 and was renewed for an additional term in June 2014 until 2017.

BioZone's strategic direction and day-to-day operations are overseen by the Centre's Executive Committee (ECOMM), consisting of the Director, three Associate Directors and the Executive Director. Membership in the ECOMM rotates amongst BioZone professors. The current membership and the related areas of responsibility are shown in the above organizational chart (Figure 7).

The ECOMM meets monthly to review new opportunities, provide an organizational framework for students and researchers, and oversee lab and computational resources. The committee also works closely with BioZone staff, as shown in the chart above, to ensure that researchers' needs are met and their concerns promptly addressed.

The BioZone Experience

BioZone provides its members with a diverse, relevant and multidisciplinary learning experience. This is manifested through coursework, teaching, and research, as well as through a variety of events.

Innovation in Education

Graduate and senior undergraduate education continues to be a focus of BioZone, particularly emphasizing a hands-on, experiential approach. Graduate students have access to a variety of courses offered through many departments within the Faculty of Applied Science and Engineering, and throughout the University, particularly in Cell and Systems Biology and Biochemistry. At the graduate level, BioZone professors offer courses that introduce *Microbial Ecology and Bioenergetics* (JCC1313: Environmental Microbiology – Edwards/Master), and build on these concepts with *Genomics and Proteomics* (CHE1135: Advances in Bioengineering - Savchenko/Yakunin), *Modeling Optimization of Chemical and Biological Networks* (CHE1125; Mahadevan) and *Liquid Biofuels* (CHE1123; Saville). At the undergraduate level, bioengineering concepts and experimentation are shared through courses offered in Environmental, Bioengineering and Biomedical Engineering minors as well as through undergraduate thesis projects.

BioZone's laboratory equipment and genomics-enabled research approaches are being incorporated wherever possible into graduate and undergraduate courses and workshops. For example, an 80L fermenter was installed in the Unit Operations Laboratory in the Wallberg Building. This reactor is being used for research, and also for teaching fermentation to senior undergraduates in CHE450 (Bioprocess Technology and Design). BioZone's Mass Spectrometry Facility has been featured already in several graduate courses, and the staff in the Mass Spectrometry Facility have piloted modular training courses on the use of mass spectrometry in bioengineering research, with the plan to develop these into formal courses for users.

The remarkable experience and talents of our dedicated staff, including research associates, lab managers and technicians (see Figures 5 and 7) are a unique resource who selflessly contribute to student training. For example, they offer small groups of students training on protocols for specific equipment, or on the use and maintenance of precision syringes and other lab equipment, protocols for DNA, RNA or protein extraction, PCR, and a variety of specialized software. These experienced research staff work alongside students and postdocs, providing continuity and consistency in training, and assistance with large grant preparation and reporting. In addition, all BioZone students rotate on a monthly basis through a lab duty roster and are responsible for the maintenance of specific instruments and training of new recruits.

Students and staff are also encouraged to participate in non-technical professional development. In the past, we have run workshops on visual and public communication of science and engineering, and on the use of Adobe Illustrator for creating attractive figures and on basic elements of design and audience engagement. We continue to promote communication through the inauguration of the BioZone newsletter, which was conceived and produced by a team of students and staff (https://www.biozone.utoronto.ca/newsletter-archive/).

International Exchanges

As in previous years, we continued to build on the many domestic and international collaborations previously established by our researchers. BioZone hosted a number of international visitors and collaborators including:

- one M.Sc., three Ph.D students and one postdoctoral research fellow from Aalto University (Finland)
- one postdoctoral researcher from the Chinese Academy of Agricultural Sciences (China)
- a visiting industry researcher from DuPont
- a visiting student researcher and a professor from the Federal University of Pernambuco (Brazil)
- a Ph.D. student from Jiangnan University (China)
- an M.Sc. student from Memorial University (Newfoundland, Canada)
- a Ph.D. student from Nanjing University (China)
- a professor from Northeast Agricultural University (China)
- a Ph.D. student from the Swedish University of Agricultural Sciences (Sweden)
- a professor from Tianjin University (China)
- a Ph.D. student from Tsinghua University (China)
- a Ph.D. student from the Universidade Federal de São Paulo (Brazil)
- two postdoctoral research fellows from the University of Calgary (Alberta, Canada)
- a postdoctoral researcher from the University of Guelph (Ontario, Canada)
- a professor from the University of Helsinki (Finland)
- a visiting Ph.D. student from the University of Tartu (Estonia)
- a graduate student from the University of French West Indies (Guadeloupe)
- one M.Sc. and one Ph.D. student from the University of Western Ontario (Ontario, Canada)
- an assistant professor from Chejiang University (China)

During this time, BioZone sent:

- Professor Elizabeth Edwards to São Paolo for ongoing collaborations with Brazilian researchers
- Professor Emma Master to Finland as a part of her research grant from the European Research Council

Research Accomplishments

Awards

The outstanding work accomplished by BioZone's students and researchers was recognized with over 60 awards and scholarships. The following list highlights just a few of the awards received by BioZone's professors, post-docs and students:

- Canada Research Chair
- CIHR Training Program in Regenerative Medicine Graduate Fellowship Award
- Dr. Goran Enhorning Award in Pulmonary Research
- Dr. Joe A. Connolly Memorial Award
- Eco-Tec Founder's Scholarship
- Eric David Baker Krause Graduate Fellowship
- Erwin Edward Hart Professor in Chemical Engineering and Applied Chemistry
- Fellow of the Royal Society of Canada
- Helen L. Cross Memorial Graduate Scholarship
- Killam Prize
- McLaughlin Foundation Award for MD/PhD Students
- Mitacs Accelerate Fellowship
- Mitacs Globalink Graduate Fellowship
- NSERC Canada Graduate Scholarships - Master's Program
- NSERC CREATE in Manufacturing, Materials and Mimetics (M3)
- NSERC Postgraduate Scholarships-Doctoral Program
- NSERC Undergraduate Student Research Awards
- Ontario Graduate Scholarship
- Ontario Trillium Scholarship
- Paul Cadario Doctoral Fellowship in Global Engineering
- Professor William F. Graydon Memorial Graduate Fellowship
- Queen Elizabeth II Graduate Scholarship in Science & Technology
- William and Dorothy Palm Graduate Scholarship in Science and Technology

See "Grants, Awards & Scholarships" on page 52 for a full detailed lists.

Publications

From September 2015 to August 2016, our team published nearly 50 peer-reviewed articles in international journals. In this time, our students and researchers also delivered nearly 100 invited talks, oral presentations and poster presentations at Canadian and international conferences and institutions. For a full list of all publications and presentations, including student theses, please see the *"Publications"* section on pg. 42.

Supporting Technology Transfer

Our goal is to provide benefits to society and the environment through the development of technology that addresses important problems. To help achieve this goal, the real-world application of technologies developed in our labs is guided by a Commercialization Committee (CCOM) including external private sector, policy and academic advisors.

The CCOM helps us to strengthen ties with industry, government, and other potential end users of the knowledge, tools, and technologies developed in our labs. The CCOM also assists with identifying and acting on commercialization opportunities, as well as large-scale funding opportunities in the public and private sectors.

In the latest reporting period, BioZone researchers made six invention disclosures, filed five patent applications, and founded three companies.

We have long-standing, strong research partnerships with several corporations (see "BioZone Sponsors" on pg. 61) and are actively seeking new partners for collaborative research. In addition, many of our alumni still maintain close ties to our research, providing another conduit for technology transfer through their careers and other endeavours.

Building Momentum

Our productivity in terms of high-impact publications, presentations and international collaborations continues to grow. Research is humming along, buoyed by our new facilities and equipment, which are readily accessible and fully supported with high-calibre training.

Current efforts are focused on growing relationships with existing and new collaborators at all levels, from visiting researchers to industrial partners. Inquiries are welcome from all. Please drop by for a visit any time!

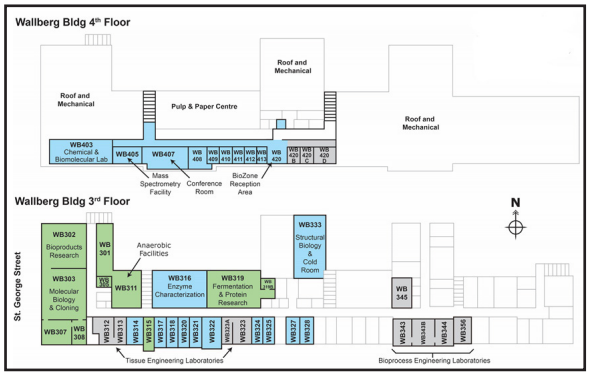
State-of-the-Art Facilities

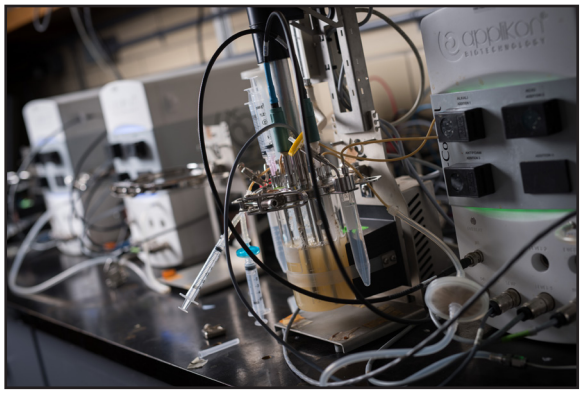
BioZone's facilities provide a collaborative space and cross-disciplinary approach that enable researchers and industry to share knowledge, procedures and equipment as they tackle difficult technical problems in environment, energy and health.

Facilities are located in the Wallberg Building at the University of Toronto and provide over 1,800 m^2 of laboratory and research workspace, including several large, bright, collaborative research labs.

The labs house a wide array of state-of-the-art analytical instruments for molecular biology, protein purification and identification, enzyme kinetics, substrate and metabolite analysis, microscopy and cell growth.

The diversity of research within BioZone provides exposure to a wide range of expertise and analyses that can be useful for any given project, arming students and researchers with a broad array of state-of-the art equipment to tackle complex problems.





Highlighted on the following pages are four of our state-of-the art facilities with specialized capabilities focusing on:

- **Mass Spectrometry**
- **Biomanufacturing**
- **Microscopy**
- **Protein Crystallization**

For more information on the other equipment and facilities available at BioZone, please visit https://www.biozone.utoronto.ca/featured-equipment/

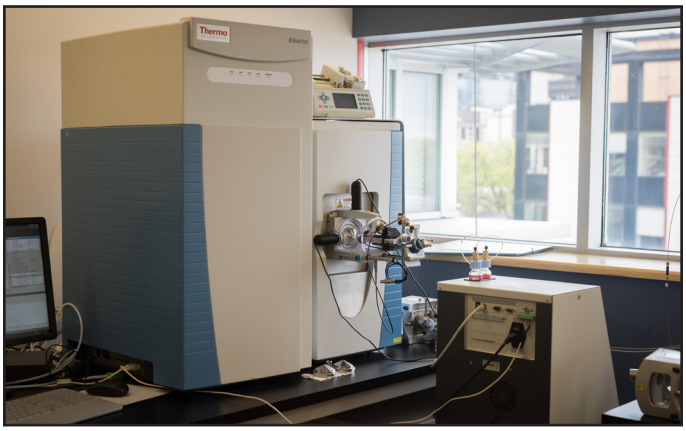
Mass Spectrometry

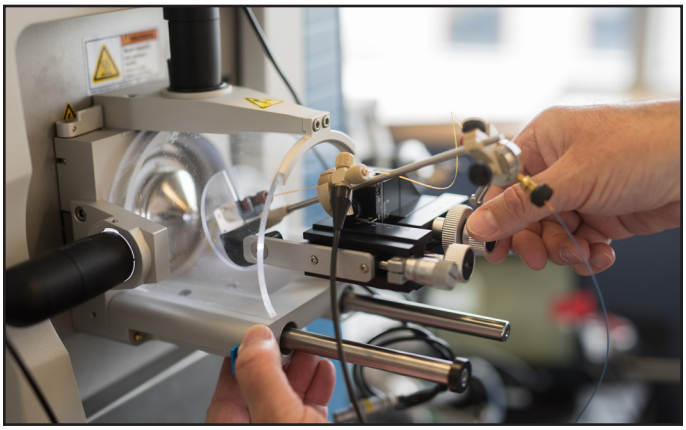
BioZone's Mass Spectrometry Facility (BioZone-MSF) operates as an analytical instrument facility serving both academic researchers and industrial clients. While the BioZone-MSF operates on a cost-recovery model, the mission of the facility is the development and implementation of novel mass spectrometry protocols for complex and difficult-to-measure samples.

This mission differentiates the BioZone-MSF from most commercial MS facilities which provide limited services based on established experimental platforms. Additionally, the BioZone-MSF places particular focus on expanding the knowledge and understanding of mass spectrometry users through education and student training.

Initially founded in 2012 with CFI funding, the BioZone-MSF has attracted a variety of users from Chemical Engineering and Applied Chemistry research groups, the larger U of T community and external researchers and companies.

The facility is operated by Facility Manager Andrei Starostine and Metabolomics Specialist Robert Flick.





Services offered by the BioZone-MSF include:

Small Molecule Analysis
- Accurate mass determination and/or quantification of analytes in a given sample. Samples are analyzed, identified, and validated through comparison to chemical standards. Examples include but are not limited to drug compounds, amino acids, vitamins, and short biopolymers.

Metabolomics
- Measurement of unique "chemical fingerprint(s)" resulting from cellular processes in an organism. By observing changes in this fingerprint as a result of cellular perturbations (deletion, insertion, mutation, external stress), we can gain a better understanding of an organism's biological mechanisms and associated phenotypes.

Proteomics
- The large-scale study of proteins produced in a given organism or cellular system. By monitoring changes in expression, co-translational and post-translational modifications, and interactions, we can gain a better understanding of an organism's cellular processes. Employing a "bottom-up" approach, we can identify and validate protein identity following proteolytic cleavage.

Sample Preparation
- To facilitate analysis, samples must be prepared in a manner suitable for mass spectrometry-based detection. Consultation and/or on-site preparation with regards to sample extraction and preparation is offered. This may involve growth and isolation of cells, quenching of cellular processes, analyte extraction and processing for mass spectrometry analysis.

Method Development
- Due to the variety of analytes that can be observed through mass spectrometry, specialized extraction, separation, and detection methods may be required. Our facility offers the development of custom methods to meet client requirements following consultation.

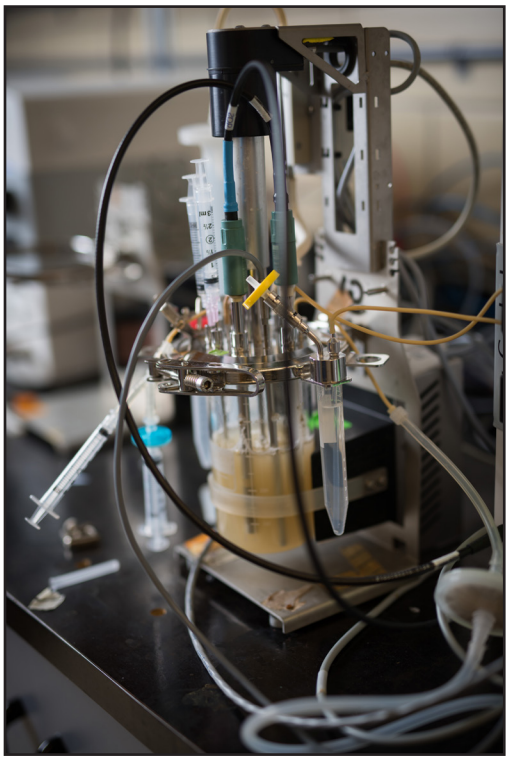
Instrumentation in the BioZone-MSF includes:
- Thermo Q-Exactive LC/MS - Hybrid Quadrupole-Orbitrap™ mass spectrometer
- Thermo Exactive LC/MS - Orbitrap™ mass spectrometer
- TOFWERK IMS-TOF- Ion mobility spectrometer (DTIMS) coupled to a high-speed time-of-flight mass spectrometer
- Thermo LTQ-XL LC/MS- Linear trap Quadrupole mass spectrometer
- Varian Saturn 2100T GC/MS- Electron Ionization equipped mass spectrometer

Biomanufacturing

Biomanufacturing, or the production of chemicals and materials using microbial fermentation, is playing an increasingly important role in the chemical and manufacturing industry. BioZone's ability to engineer and model biological systems opens avenues to produce novel and non-natural products. In doing so, BioZone embodies scale-up at an early stage, helping to ensure target strains will be commercially viable. BioZone is making further investments in biomanufacturing capacity to facilitate fee-for-service use by academic and industrial collaborators.

BioZone has a unique set of skills and tools for assessment of biomanufacturing processes from the microliter to 100L scale. Our liquid handling robotic and integrated plate reader platform allows us to perform high-throughput screening of up to hundreds of samples per day. This enables the assessment of a large number of variables from process conditions to medium composition, using appropriate experimental designs and response surface methodology.

Scaling-up, we have six 500mL bioreactors, fully equipped with mass flow controllers, pH probes and sensitive DO probes to enable precise control. From here, strains can be scaled to one of three 5L Infors bioreactors, or moved directly to 80L scale.



In addition to production vessels, we are fully equipped with state-of-the-art analytics, both offline and online. Our PrimaDB mass spectrometer allows on-line monitoring of CO_2, O_2 and volatiles such as ethanol – giving us unique insight into our process. Off-line measurements are typically performed using either HPLC or LC-MS, to give us a full range of analytical capabilities.

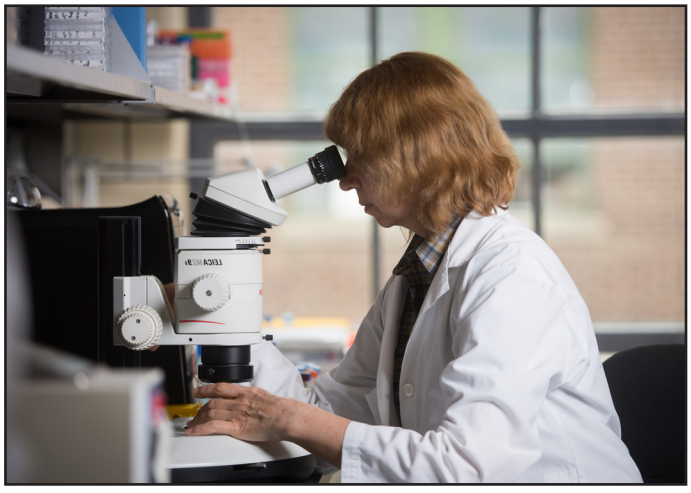
The bioreactor and analytical equipment, combined with the expertise available at BioZone in synthetic biology, metabolic engineering, and genome-sale modeling gives us a strong advantage in development and scale-up of organisms and processes for biomanufacturing.

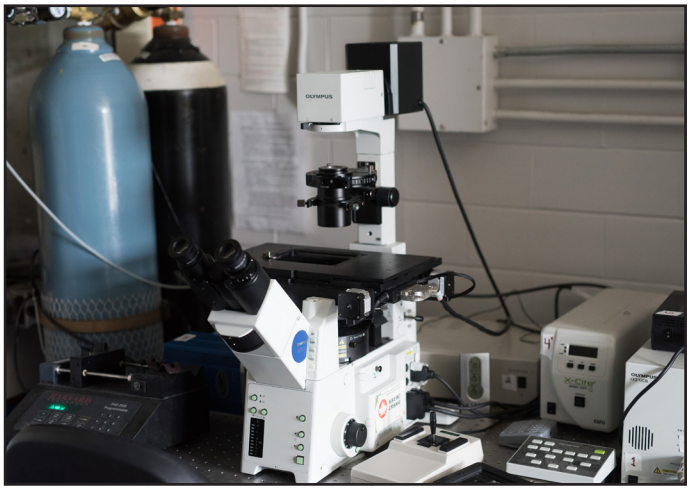
Microscopy

BioZone houses both upright and inverted microscopes that can take bright field and fluorescence images. The automated microscope also has a stage top incubator to allow imaging of cells during cell culture at 37°C for up to 5 days. Furthermore, the automated microscope has a fully motorized stage allowing image stitching of images from adjacent fields of view. Microscopes are equipped with 4X, 10X, 20X, 40X, 63X and 100X lenses. In addition, the *ImageXpress Micro* is a high content screening microscope with environmental control. Images can be automatically collected in multiple locations within multiple wells of a well plate over time under conditions that allow cells to culture. The microscope models we currently carry in BioZone include:

- Olympus CKX1 live cell inverted microscope
- Olympus 120Q live cell inverted microscope
- Olympus BX51 microscope
- Leitz Laborlux S microscope
- Molecular Devices ImageXpress Micro Widefield High Content Screening System

Examples of current projects in BioZone that utilize the microscope facilities include: quantifying the alignment response of airway epithelial cells cultured on substrates containing guidance signals, characterizing the cooperative behaviour of epithelial cells re-organizing in confluent 2D monolayer sheets, assessing cellular invasion of cancer cells into surrounding stromal tissues in 3D, and real-time monitoring of engineered tumor growth in response to drug treatments.



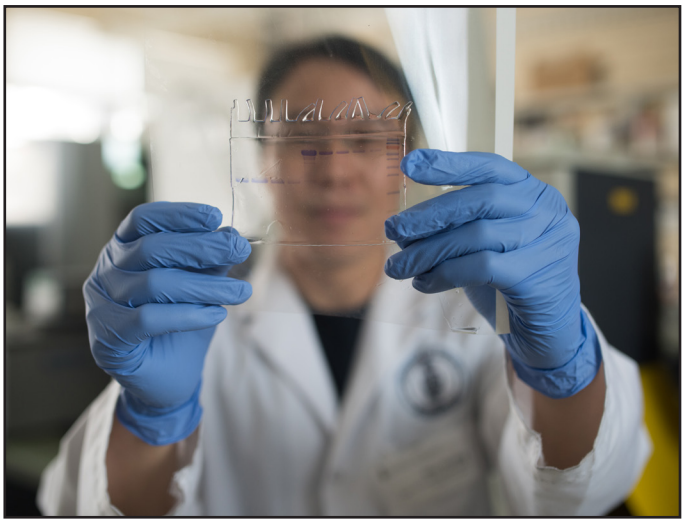


Protein Production, Screening & Crystallization

The BioZone Protein Production and Crystallization Facility specializes in:

- cloning
- protein production
- protein purification
- enzyme functional characterization
- protein 3D structural characterization

These services allow users to: assign function to genes, determine the molecular basis for substrate recognition, identify the roles of sequence motifs in enzyme function, determine how to inhibit enzyme function, and tailor the properties (i.e., specificity, stability, catalytic rate) of enzymes.



The Facility houses:

- protein purification and crystallization equipment
- chromatography/FPLC systems
- Mosquito crystallization robot
- specialized crystallization reagent screens
- Rigaku Micromax-007 x-ray diffractometer system

In addition to advanced instrumentation, the facility houses thousands of cloned genes and characterized enzymes and a highly experienced, specialized, and collaborative team with 40+ years of collective experience in molecular structure determination and interpretation.

BioZone Researchers

BioZone's strength resides in its people. We are home to a talented group of over 100 highly qualified personnel and trainees who bring a wide range of expertise and experience to their work, and tackle challenging research goals in a supportive and collegial community.

The following pages include profiles of BioZone members:

Principal Investigators: **Page 26**
Researchers: **Page 35**
Research Support Staff: **Page 36**
Undergraduate Experience: **Page 38**
Alumni: **Page 39**

Professor D. Grant Allen

Environmental bioprocess engineering for the treatment of wastewater, air quality, and the conversion of waste into value-added products

Dr. Grant Allen is a Professor in the Dept. of Chemical Engineering and Applied Chemistry and began serving as Department Chair in July 2011. He previously served as Vice-Dean (Undergraduate) for the Faculty of Applied Science and Engineering, the Department's Associate Chair for Graduate Studies (2003-2007) and Director of the Pulp and Paper Centre (2001-2003).

Dr. Allen completed his Ph.D. in Chemical Engineering at the University of Waterloo in 1987. His many professional accomplishments include: Fellow of the Chemical Institute of Canada, the Canadian Academy of Engineering, the Engineering Institute of Canada and the American Association for the Advancement of Science.

He has published over 100 refereed publications and has led research consortia involving international partners from Brazil, Japan, New Zealand and the USA. He received the LeSueur Memorial Award in developing technical excellence from the Chemical Institute of Canada in 2014.

Research Description

Dr. Allen's research focuses on the interdisciplinary field of bioprocess engineering. He investigates bio-processes that utilize microorganisms to treat industrial wastes and convert them into value added products. His research has included several aspects including understanding and optimizing biological wastewater treatment systems for treating chlorinated organic compounds and managing process disturbances (e.g., temperature, oxygen, concentration. He has also conducted extensive research on the biological treatment of waste gases. Most of his work has been associated with the pulp and paper industry.

Dr. Allen's current research focuses on two areas. The first involves enhancing the management of biosolids produced by industrial treatment systems including anaerobic treatment, dewatering processes and producing value-added products. His second area of research involves the development of biofilm photobioreactors and processes to grow microalgae for fuels/chemicals from carbon dioxide, sunlight and wastewater.

Applications

- Industrial wastewater treatment (i.e., pulp and paper industry)
- Conversation of CO_2 and wastewater biomass to value-added chemicals and fuels
- Optimization and modeling of bioprocesses
- Biosolids dewatering
- Green bioflocculants

Expertise & Impact

Dr. Allen's focus on bioprocess engineering allows his work to integrate advances from a wide range of collaborators including engineers, microbiologists, biologists, and chemists into applied technologies that function effectively in industrial settings including: process modeling, bioreactor design, molecular analytical tools, green technologies, bioproducts and environmental engineering.

Ongoing Partnerships: Biox Corporation, Mara Renewables, Pond Biofuels, GE Water, Andritz, Arauco, AV Group NB, CENIBRA, Clyde-Bergemann Power Group, Daishowa-Marubeni International, Domtar, Eldorado Brasil, ERCO Worldwide, Fibria, FPInnovations, Georgia-Pacific, International Paper, Irving Pulp & Paper, Kiln Flame Systems, Klabin, Stora Enso, Suzano Papele Celulose, Tembec, Tolko Industries, Valmet, WestRock

Professor Levente L. Diosady

Prevention of micronutrient deficiency diseases through the development of process technology that fortifies food with micronutrients

Dr. Levente Diosady is a Professor in the Dept. of Chemical Engineering and Applied Chemistry at the University of Toronto, where he directs the Food Engineering Group. He received his Ph.D. from the University of Toronto in 1971. He is a Fellow of the Chemical Institute of Canada, Canadian Institute of Food Science and Technology, Canadian Academy of Engineering, the Hungarian Academy of Engineering, the International Academy of Food Science and Technology, the American Oil Chemists' Society and the Royal Society of Canada.

Dr. Diosady's innovative work has been recognized with many professional awards, including the Babcock-Hart Award from the Institute of Food Technologists honouring an IFT member who has contributed to improved public health through nutrition. Dr. Diosady is the author of over 130 refereed publications and 22 patents. His many honours include the Order of Ontario (2010), the Queen's Diamond Jubilee Medal (2012) and the Order of Merit of Hungary (2015).

Research Description

The Food Engineering Group adapts the unit operations of traditional chemical engineering to the specific requirements of food processing. Specifically, processes that allow for the addition of important micronutrients, such as iron, iodine, folic acid, zinc, and vitamin A, to common foods such as tea, salt, and rice in a way that does not affect food taste, colour, smell or texture. The cost-effective addition of these nutrients to inexpensive foods can prevent millions of people in developing countries from developing crippling diseases such as anemia or blindness.

The processing of vegetable oils is another active area of research in the Food Engineering Group. They have developed a process for the production of protein isolates from canola, rapeseed and mustard seed and are working on processes that will recover food grade protein, and high-quality biodiesel from a number of oilseeds and algae, thus contributing both fuel and food from crops that are now unused, or have marginal utility. In addition, the Food Engineering Group is developing novel catalytic processes that reduce the negative impact oils have on cardiac health by minimizing the formation of trans double bonds but retain the desirable characteristics in the oil.

Ongoing Partnerships: JVS Foods PvC - Jaipur, Nutrition Impact Solutions Inc., G.S. Dunn Mustard Millers Inc., WHINCORP, et al.

Applications

- One third of the world's population is suffering from inadequate intake of micronutrients, which interferes with the physical and mental development of children, results in reduced immunity to disease, reduced work capacity, increased blindness and mortality especially amongst women and children. By fortifying low cost foods with iodine and iron in combination with folic acid, zinc or vitamin A, the prevalence of these diseases can be significantly reduced.
- Creation of healthier foods through the creation of vegetable oils with fewer trans fats.
- Recovery of food quality protein from oils and of oilseeds and algae used for biofuel production.

Expertise & Impact

The Food Engineering Group works with national and international agencies, including IDRC, Grand Challenges Canada and the Bill and Melinda Gates Foundation, to develop technologies for food fortification. The technology, for the simultaneous fortification of salt with iron and iodine, has been pilot-tested in India, where more than one million children have been cured of anemia by school lunches cooked with double-fortified salt, and more than 24 million people are now consuming double-fortified salt.

Professor Elizabeth Edwards

Utilizing the latest genomics and metagenomics tools to understand and exploit microbial communities that detoxify common pollutants, convert wastes into useful products, and to minimize solid waste streams

Dr. Elizabeth Edwards is the Director of BioZone and is a Professor in the Dept. of Chemical Engineering and Applied Chemistry. She holds Bachelor's and Master's degrees in Chemical Engineering from McGill University and a Ph.D. in Environmental Engineering and Science from Stanford University.

Dr. Edwards' research accomplishments have been recognized with an NSERC Women's Faculty Award, a Premier's Research Excellence Award, a Killam Research Fellowship (Canada Council for the Arts), an Ontario Professional Engineers Award and in 2016, she was awarded the prestigious Killam Prize (Canada Council for the Arts). She has been inducted into the Canadian Academy of Engineering (2011) and is a Fellow of the Royal Society of Canada (2012). Dr. Edwards currently holds the Canada Research Chair in Anaerobic Biotechnology.

Research Description

The focus of Dr. Edwards' work is to harness and enhance the innate ability of anaerobic microbes to biologically transform common toxic pollutants or to convert waste to more valuable products. Her research involves the molecular, phylogenetic, and physiological characterization of microbial communities to improve biochemical reaction rates. Dr. Edwards' group has a long history of working with microbial communities that can detoxify chlorinated (e.g., PCE) and BTEX compounds.

In addition to bioremediation, Dr. Edwards' work involves the study of anaerobic digestion of waste streams. Anaerobic digestion of organic waste has tremendous potential to address the economic and environmental pressures facing most industries and municipalities. While anaerobic digestion is widely applied already in certain sectors, there is keen interest to expand its use to new waste streams (e.g., the pulp and paper industry). Using a combination of pre-treatment methods and reactor configurations with new molecular and analytical tools to gain mechanistic information on anaerobic processes, her lab is investigating alternative approaches to recover energy from waste liquid and solid streams.

Applications

- Bioremediation and bioaugmentation of sites contaminated with chlorinated compounds, BTEX, and other pollutants.
- Reduction of industrial and municipal waste solids through anaerobic digestion processes.

Expertise & Impact

Dr. Edwards brings expertise in engineering scale-up and commercial application of bioproducts to BioZone, and was recognized with the 2009 NSERC Synergy Award for her highly successful partnership with GeoSyntec, an international environmental consulting firm with whom she developed a microbial consortium called KB-1®. This commercially successful bioproduct biodegrades two of the world's most common and persistent industrial groundwater pollutants: PCE (a common dry-cleaning agent) and TCE (a degreasing solvent), more quickly and at a lower cost than conventional methods. It has been used at over 300 sites around the world.

Ongoing Partnerships: SiREM, GeoSyntec, DuPont, Dow, Suncor, BP, Tembec

Professor Radhakrishnan Mahadevan

Engineering microbial systems for industrial and medical applications by employing bioinformatics, modeling of metabolic and regulatory networks, systems biology, and metabolic engineering

Dr. Radhakrishnan Mahadevan is an Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry. He is cross-appointed to the Institute of Biomaterials and Biomedical Engineering and holds a Ph.D. in Chemical Engineering from the University of Delaware. Prior to joining the University of Toronto in 2006, he completed a postdoctoral fellowship at Genomatica Inc., and was a visiting researcher at the Universities of Massachusetts and California in San Diego.

Dr. Mahadevan's research has been published in nearly 80 peer-reviewed publications including publications in ISME, Chemical Sciences, Metabolic Engineering and Nature Reviews Microbiology. He has received many accolades including the Society of Industrial Microbiology and Biotechnology Young Investigator Award (2012), the University of Toronto FASE Research Leaders Award (2013), the Alexander von Humboldt Fellowship (2013) and the Syncrude Canada Innovation Award (2014).

Research Description

Dr. Mahadevan is a pioneer in the fields of systems biology, synthetic biology, and constraint-based models of metabolic networks. His research interests include systems analysis, engineering and control of biological processes, and genome-scale models of cellular processes. This expertise allows for the design of dynamic model-driven engineering strategies for biological process optimization and control across different length and time scales.

Applications

A focus of Dr. Mahadevan's work is designing cells to sustainably and economically produce a wide range of high-value chemical products from renewable feedstocks such as agriculture and forestry waste streams. Chemicals produced biologically feature lower toxicity and greenhouse gas emissions than traditional petrochemical systems. Other applications of his work include optimization of bioremediation strategies, engineering microbial fuel cells (increasing current), biomedical engineering (drug design and dosage, personalized nutrition and medicine), bioreactor control and optimization (designing optimal substrate and inducer feeding strategies), and bioremediation (determining the spatiotemporal substrate addition strategies to effectively stimulate microbial activity).

Expertise & Impact

His group has developed a suite of novel computational and experimental tools for metabolic engineering including bioinformatics, metabolic modeling and other systems biology tools, genetic circuits such as toggle switches and other synthetic biology elements. Dr. Mahadevan's group has engineered cells to efficiently produce chemicals such as adipic acid for bionylon production, 1,3-butanediol, ribose, and glycolic acid from renewable feedstocks. This intellectual property has resulted in several startup companies and collaborations with renewable chemical companies. In addition to biochemicals, the group also applies metabolic modeling and engineering for analyzing the metabolic interactions in microbial communities relevant to bioremediation, gut microbiome, and has developed multi-scale models of whole human body metabolism.

Ongoing Partnerships: BioAmber, FPInnovations, Suncor, DuPont, Lallemand

Professor Emma Master

The implementation of enzymology, protein engineering, proteomics, applied functional genomics, and lignocellulose chemistry for the production of high-value products from renewable plant resources

Dr. Emma Master is a Professor in the Dept. of Chemical Engineering and Applied Chemistry and is BioZone's Associate Director for Laboratories and Facilities. She holds a Ph.D. in Environmental Microbiology from the University of British Columbia and completed postdoctoral fellowships at the Royal Institute of Technology in Stockholm and Concordia University before joining the University of Toronto in 2006. She is cross-appointed to the Department of Cell and Systems Biology.

Dr. Master received a Basmadjian Teaching Effectiveness Award (2014, 2016), a Faculty Research Leader Award (2013), a Finnish Distinguished Fellowship (FiDiPro) (2010) and an Early Researcher Award from the Ontario Ministry of Research and Innovation (2009). Dr. Master has published 58 peer-reviewed articles and is currently leading an ERC Consolidator Grant in Aalto University (Finland) and heading the four-year Synbiomics project funded by Genome Canada.

Research Description

The aim of Dr. Master's research program is to harness recent advances in life science technology to discover and develop enzymes and non-catalytic proteins that can be used to sustainably synthesize high-value chemicals and polymers from renewable plant resources. Given the complexity and highly functionalized chemistry of plant macromolecules (e.g., hemicellulose and lignin), the reactive specificity of many enzymes is particularly valuable in the development of new bio-based materials that have tailored and reproducible performance. In particular, her approach to developing high-value products from plants benefits from two key advantages of many bioprocesses: 1) catalytic specificity that is critical for the modification of heterogeneous plant polymers and, 2) comparatively mild reaction requirements that retain valuable properties of starting plant materials (e.g., degree of polymerization). By learning how to better harness renewable plant resources, we can sustainably supply the global market for high-value biochemical and bioplastics while finding new markets for Canada's forest and agricultural sectors, which are vital to Canada's economy and to further support small communities across our country.

Ongoing Partnerships: EcoSynthetix, IGPC, Gruber, DuPont, Tembec

Applications

A focus of Dr. Master's work is developing cell-free, chemo-enzymatic pathways that sustainably produce a wide range of high-value chemicals and materials from renewable plant resources that are typically produced using toxic and environmentally harmful petrochemicals. These include non-formaldehyde binders, bioplastics, adhesives, solvents, biosurfactants, and emulsifiers.

Expertise & Impact

Dr. Master's team has harnessed the regio- and stereo-specificity of enzymatic reactions, and the versatility of chemical catalyses, to create chemo-enzymatic pathways that build upon the intrinsic value of plant-derived polymers. Her group has also established several complementary surface analysis methods to discover and optimize chemo-enzymatic pathways that diversify the range of renewable biomaterials made from plant biomass. Dr. Master's focus on: 1) high-value products with existing market pull, 2) application of under used lignocellulose fractions, and 3) development of biotechnologies that are scalable and readily distributed, is expected to expand Canada's role in global bioproduct markets while creating lasting knowledge-based economic opportunities for Canada's forest and agricultural sectors.

Professor Alison McGuigan

The development of predictive structured heterogeneous tissue model systems for understanding mechanisms of tissue assembly and to improve drug discovery

Dr. Alison McGuigan is an Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry. She joined the department in 2009 after receiving her Ph.D. in Chemical Engineering at the University of Toronto and completing postdoctoral fellowships at Harvard University and Stanford Medical School. She is cross-appointed to the Institute of Biomaterials and Biomedical Engineering.

Dr. McGuigan's innovative research into assembling engineered tissues has been recognized with the Wake Forest Institute for Regenerative Medicine Young Investigator Award (2008) and the Tissue Engineering and Regenerative Medicine International Society Young Investigator Award (2013). She has authored 36 refereed publications, and her research is funded by NSERC, CIHR and Medicine by Design.

Research Description

Cell behaviour in standard 2D model experimental systems is often not predictive of real life response. It is therefore important to develop physiologically relevant, personalized, 3D tissue model systems. These model systems offer the opportunity to systematically dissect fundamental mechanisms of tissue assembly and disease and improve the effectiveness of drug discovery and enable the design of personalized therapies.

The McGuigan group develops: 1) technologies to control cell organization at the tissue and cellular scale and, 2) strategies to stratify and visualize complex data sets from these heterogeneous tissue systems. Using these platforms, they are exploring the rules of tissue self-assembly, mechanisms of disease and the development of novel therapies.

Her group's tissue modeling includes:

- Assembly of tumour tissue microenvironments that recapitulates specific characteristics of tumours, such as the presence of oxygen gradients or the surrounding stroma.
- Epithelial microenvironment engineering that subject progenitor cells and airway epithelial cells that model mechanical and architectural stimuli to influence cell fate choices.
- Tissue patterning that models the geometry and patterning in tissues containing two or more cell types.
- Analysis pipeline that assess individual cell or group migrations in confluent.

Applications

- Realistic 3D tumour models for testing cancer therapeutics
- Airway epithelium tracheal resection and reconstruction
- Recapitulating the architecture of native tissue for testing and therapeutics

Expertise & Impact

Professor McGuigan's group has expertise in:

- Tissue engineering
- Tissue culture
- Microscopy
- Visualization
- Imaging

Ongoing Partnerships: Johnson & Johnson, Princess Margaret Cancer Centre

Protein Production & Crystallization

Professor Alexei Savchenko

Characterization of protein function based on combination of structural, biochemical and in vivo *methodologies*

Dr. Alexei Savchenko is a former Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry. He was also an Assistant Professor in the Banting and Best Dept. of Medical Research and is Group Leader at Structural Proteomics in Toronto (SPiT). Dr. Savchenko currently serves as an Associate Professor at the University of Calgary in the Department of Microbiology, Immunology and Infectious Diseases and an Adjunct Associate Professor, Department of Biochemistry and Molecular Biology as of September 2016. He received a Ph.D. in Molecular Biology and Microbiology from the University of Nantes in 1996 and joined the University of Toronto in 1999 after completing a postdoctoral fellowship in protein biochemistry at Michigan State University.

While Dr. Savchenko has moved onto the University of Calgary, he still leads a research team of graduate students, laboratory technicians, postdoctoral fellows and research associates in BioZone. Dr. Savchenko has published nearly 150 papers and reviews, and his U of T research is currently funded by NIH, CIHR, the Ontario Research Fund and NSERC.

Research Description

The Protein Production and Crystallization Facility studies protein functional mechanisms using a combination of structural (primarily X-ray crystallography), biochemical and *in vivo* methodologies. The group works on large-scale structural characterization of protein families with key biological or biocatalytic roles. Dr. Savchenko and his team developed the high-throughput protein crystallization pipeline that is central to major international structural genomics initiatives. This pipeline produces more than 80 novel protein structures per year and is one of the most efficient in the field of structural biology. In collaboration with Dr. Alexander Yakunin, Dr. Savchenko has done pioneering work in enzyme discovery and biochemical characterization of novel microbial enzymes. Ongoing research avenues include molecular characterization and inhibition of enzymes conferring antibiotic resistance and bacterial pathogenesis factors.

Applications

Since the 3D structure of an enzyme is matched to its molecular function, visualization of the 3D structure of an enzyme allows for deeper understanding of its biomolecular function. This includes: assigning a catalytic activity to a gene sequence, understanding the molecular basis of ligand binding, catalysis and active site properties, guiding of experiments including mutagenesis and activity screening and, avenues for rational engineering such as alteration of ligand specificity. Research advances into the 3D structures of enzymes by the Savchenko group have advanced knowledge of various biocatalytic processes such as production of polymeric building blocks and carbohydrate degradation, infectious mechanisms of bacterial pathogens, and drug discovery for treatment of the bacterial antibiotic resistance. Overall, this research aids in rational deployment and engineering of enzymes for biocatalytic applications and in the inhibition of enzymes for the treatment of infectious disease.

Expertise & Impact

Dr. Savchenko's team are among the world's most experienced groups in structural genomics and protein crystallography. Combined, the team has more than 40 years of experience in protein purification and 3D structure determination and functional analysis. Professor Savchenko's group has expertise in: structural genomics, X-ray crystallography, protein structural analysis, high-throughput protein purification, high-throughput cloning and mutagenesis, inhibitor and drug discovery.

Ongoing Partnerships: Pasteur Institute, Northwestern University, University of Chicago, Argonne National Laboratory, Imperial College London, Tufts University, UBC and McMaster University.

Professor Bradley Saville

Conversion of biomass for production of biofuels and bioproducts, and the economic and life cycle analysis of biofuels and bioproducts

Dr. Bradley Saville is a Professor in the Department of Chemical Engineering and Applied Chemistry where he leads the Bioprocess and Enzyme Technology research group. He joined the University of Toronto in 1989 after receiving his Ph.D. in Chemical Engineering from the University of Alberta. He has authored over fifty refereed publications, four books and book chapters/monographs and over forty-five technical reports, and he holds twenty-five patents. Dr. Saville is also the founder of Savant Technical Consulting.

Research Description

Dr. Saville's research involves bioprocessing of biomass to produce biofuels and high-value bioproducts, including the design and scale-up of reactors for enzymatic hydrolysis, biomass pretreatment, and bioseparations. A particular focus is on using enzymes for conversion of starch and lignocellulosics into sugars, biofuels, and bioproducts, and on biomass pretreatment methods needed to enhance enzyme hydrolysis of lignocellulosics. Also within this subject area, they aim to develop comprehensive process models of biofuel/bioenergy production systems, for the purposes of financial analysis and life cycle assessment.

Dr. Saville's research program also involves developing methods to improve enzyme function and performance. Successful industrial use of enzymes is contingent upon the availability of stable, low-cost enzyme preparations. Some enzymes are subject to substrate-induced inactivation, whereby the exposure of the enzyme to a toxic substrate can lead to an irreversible loss of activity. The occurrence of inactivation and inhibition has significant implications upon industrial biochemical production.

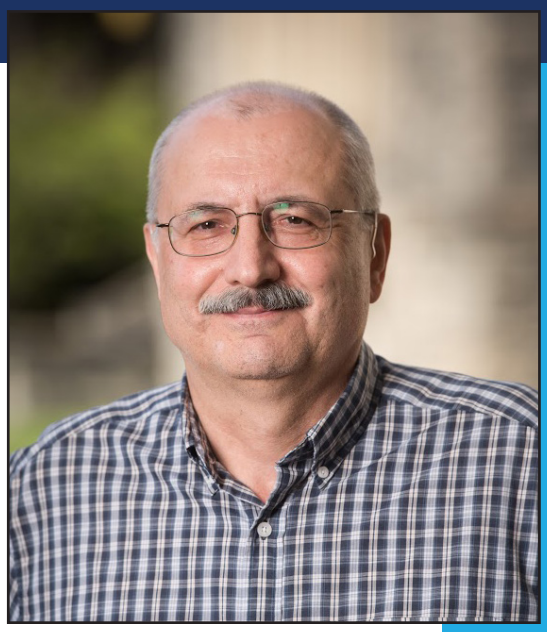
Ongoing Partnerships: Transport Canada, Canergy, Prenexus Health

Applications

Dr. Saville's areas of interest include enzymatic hydrolysis of starchy and lignocellulosic substrates for production of biofuels and bioproducts, and the characterization of hydrolytic enzymes and enzymes for biofuels and biopolymer synthesis. His enzyme work includes applications for bioremediation, pulp and paper, and pharmaceuticals. His research group is also involved in bioreactor design and scale-up, as well as processing of high-consistency biomass slurries. In addition to the technical aspects of bioproducts, Dr. Saville has also been very active in the economic and life cycle analysis of biofuels, bioenergy and bioproducts, as well as bioenergy policy.

Expertise & Impact

Dr. Saville is an expert in biofuels and bioenergy benchmarking, and has been involved in the biofuels research field ranging from bench scale R&D to commercialization, economics and policy. Technology derived from Dr. Saville's research and patents related to novel hydrolytic enzymes has been field-tested in several North American fuel ethanol plants, and in the development of lignocellulosic processes for biofuels and bioproducts. He has collaborated with numerous industry and government partners (e.g., SunOpta Bioprocess Inc., Mascoma, Transport Canada) on various projects related to biofuels, bioenergy and bioproducts.



Professor Alexander Yakunin

Discovery and biochemical characterization of novel enzymes, development of new enzymatic screens, enzyme applications in biocatalysis and bioremediation, molecular mechanisms of CRISPR

Dr. Alexander Yakunin (Iakounine) is an Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry. Dr. Yakunin completed an M.Sc. in Molecular Biology at Moscow State University, and he holds a Ph.D. in Microbiology from the Institute of Microbiology in Moscow (Russian Academy of Sciences). He was a postdoctoral fellow at the Institute of Soil Science and Photosynthesis in Pushchino (Russia) and at the University of Montreal before joining the University of Toronto in 2002.

Dr. Yakunin is currently funded through NSERC, CCSRI and the Samsung Advanced Institute of Technology. Dr. Yakunin has broad expertise in microbial physiology, enzymology, and biotechnology, and has published over 140 peer-reviewed articles.

Research Description

1. Novel Industrial Enzymes

Despite the obvious benefits of biocatalysis, the major hurdles hindering the application of enzymes are the limited repertoire of the available enzymes and their low activity and stability. Dr. Yakunin's lab is exploring the wealth of environmental metagenomes and sequenced genomes to discover new industrial enzymes for applications in biocatalysis and bioremediation. Their approach is based on the high-throughput enzymatic screening of metagenomics libraries and purified unknown proteins, as well as on biochemical and structural characterization of novel hydrolases and oxidoreductases (esterases, lipases, phosphatases, nucleases, dehydrogenases, aldo-keto reductases and oxidases).

2. Molecular mechanisms of CRISPR

It has been shown that CRISPRs and the associated Cas proteins represent a novel microbial genome defense system which complements the well-known restriction modification system, but utilize completely different mechanisms. The Cas proteins comprise up to 45 families of predicted nucleases, many of which are expected to be sequence- or structure-specific. The biochemical and structural studies of the Cas proteins will help unveil the CRISPR mechanism and reveal new enzymes for genetic engineering.

Ongoing Partnerships: DuPont, Samsung

Applications

- Identification and optimization of enzymes for the bioconversion of renewable feedstocks to valuable chemicals, fuels, polymers, and other products. This will allow industries to reduce their carbon emissions and reliance on oil and toxic compounds.
- Identification and optimization of enzymes for waste biodegradation and bioconversion to fuels
- Enzyme discovery for bio-based plastic recycling
- Improved enzymes and protein complexes for genome editing and genetic engineering
- Enzyme discovery for CO_2 fixation and bioconversion to valuable chemicals and fuels

Expertise & Impact

Extensive experience with a broad range of enzymes including hydrolases (esterases, lipases, phosphatases, phosphodiesterases, and nucleases), oxidoreductases (dehydrogenases, oxidases, aldo-keto reductases, enoate reductases, and carboxylate reductases), and transferases (aminotransferases and kinases). Over 20 general and specific enzymatic screens for purified proteins and metagenomics gene libraries. Collection of over 800 novel enzymes from environmental metagenomes and genomes including many enzymes for the production of commodity chemicals and plastic depolymerization.

BioZone Researchers

BioZone has grown over the years to attract the best and brightest researchers from around the world. The following includes a list of graduate students, post-doctoral research fellows, research associates and laboratory technicians as of Fall 2016.

For a more in-depth look into the ongoing research in BioZone, please refer to the *Researcher Profiles* located on page 63.

M.Sc. Students

Teresa Dean
Suzana Kraus
Elisse Magnuson
Nadia Morson
Fakhria Muhammad Razeq
Hajar Pourbafrani
Viive Sarv
Sergei Vilbik
Zhiyu (Mandy) Xu

M.A.Sc. Students

Samantha Cheung
Spencer Imbrogno
Rachel Kwan
HyunWoo (Peter) Lee
Ana Victoria Legorreta Sianez
Roman Malekzai
Richard Ndubuisi
Benjamin Slater
Johnny Xiao
Jaehoon Ya
Mitchell Zak

M.Eng. Students

Kaustubh Kadam
Burton Mendonca

Ph.D. Students

Maryam Arefmanesh
Amir Arellano Saab
Sofia Bonilla
Xu (Charlie) Chen
Zahra Choolaei
Kevin Correia
Elisa D'Arcangelo
Christian Euler
Julie-Anne Gandier
Srinath Garg
Adriana Gomez
Nigel Guilford
Mahbod Hajighasemi
Masood Khaksar Toroghi
Taeho Kim
Sofia Lemak
Camila Londono
Elisa McGee
Oluwasegun Modupe
Olivia Molenda
Kayla Nemr
Mehdi Nouraei
Jon Obnamia
Kylie O'Donnell
Aditya Vik Pandit
James Poon
Luz Puentes Jacome
Darren Rodenhizer
Fawzi Salama
Sayeh Sinichi
John Soleas
Shyam Srinivasan
Tim Sun
Dylan Valleau
Azadeh Vatandoust
Naveen Venayak
Kaushik Raj Venkatesan
Po-Hsiang (Tommy) Wang
Ruoan Wang
Mabel Wong
Ruoyu Yan

Postdoctoral Fellows

Yaser Khojasteh-Salkuyeh
Kiruba Krishnaswamy
Fei Luo
Sedric Pankras
Anubhav Pratap Singh
Andrew Quaile
Juveria Siddiqui
Caroline Vanderghem
Thu Vuong
Ivy (Minqing) Yang
Dan Zeng

Research Associates

Yaldah Azimi
Bih-King Chen
Maryam Foumani
Anna Khusnutdinova
Torsten Meyer
Peter Stogios
Weijun Wang

Technical & Research Staff

Nancy Bawa
Greg Brown
Rosa Di Leo
Elena Evdokimova
Robert Flick
Shen Guo
Line Lomheim
Ben MacCormick
Travis Oakes
Tatiana Skarina
Andrei Starostine
Bella Xu

Research Support

The research undertaken by BioZone researchers could not be accomplished without the support of administrative and technical staff. All members of our support staff have a background in science and engineering which helps in meeting the needs of a large, collaborative research group.

Dr. Sean Caffrey
Executive Director

Sean provides oversight for BioZone administrative and technical staff, ensures effective and sustainable operations, acquires funding support, maintains external stakeholder relations (partner building), and helps students and staff translate research discoveries. Sean obtained an MBA and a Ph.D. in Microbial Functionality Genomics from the University of Calgary.

Endang Susilawati
Lab Manager

Susie is an experienced researcher who joined BioZone in 2009, bringing a wealth of technical experience to the task of managing lab operations. She holds an M.Sc. in Crop and Soil Sciences from Michigan State University and was a researcher at the Indonesian Oil Palm Research Institute. Susie manages daily lab activities in BioZone and provides students and researchers with information regarding safety, training, supplies and ordering. She is responsible for the maintenance of shared equipment and student lab duties.

Tedd Konya
Project Manager

Tedd provides project management support for several of the large applications on-going at BioZone. Tedd comes to BioZone from the Dalla Lana School of Public Health, where he managed a lab in the Division of Occupational & Environmental Health. Tedd holds a B.S. in Biology (Fairleigh Dickinson University), Master of Arts in Teaching (University of Pittsburgh), Master of Public Health (UofT), and most recently completed a Certificate in Project Management (UofT).

Dean Robson
Information Technology Specialist

Dean manages BioZone's server and network infrastructure and provides IT support to BioZone's faculty, researchers, and staff. In addition to his responsibilities with BioZone, Dean also provides IT support to the Department of Chemical Engineering and Applied Chemistry. Dean has a B.Sc. from Lakehead University in Computer Science and previously worked at the University of Toronto in the Faculty of Arts & Science, IIT.

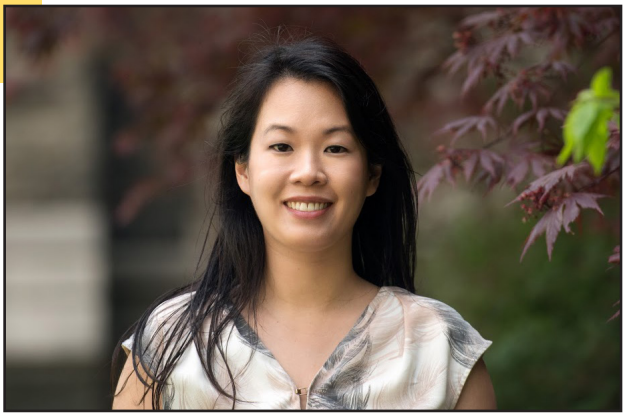
Andrei Starostine
Mass Spectrometry Facility Manager

Andrei provides technical support and expertise for the application of mass spectrometry to a wide range of biological and inorganic analyses, including high resolution protein identification and small molecules characterization. He has developed experimental methods for chromatographic separation of protein complexes and for organic low molecular structural determination. Andrei is responsible for managing the MS Facility and training students and researchers on the use of LC and GC-MS techniques in chemistry and biology.

Robert Flick
Mass Spectrometry Facility Metabolomics Specialist

Robert provides experimental and analytical support for the application of mass spectrometry ranging from high resolution protein identification to small molecule characterization and metabolomics. He has developed a variety of experimental methods for chromatographic separation and subsequent mass spectrometry-based detection in order to further the scientific research being conducted in BioZone. He operates under the BioZone Mass Spectrometry Facility providing services to researchers both affiliated and not affiliated with the University of Toronto. Robert obtained an M.Sc. in Biochemistry from the University of Toronto.

Katrina Chu
Administrative Coordinator



Katrina works on overseeing grant administration and various advancement projects within BioZone. She has a diverse science background, including an M.Sc. in river ecology from the Canadian Rivers Institute at the University of New Brunswick, and an H.B.Sc. from the University of Toronto in evolutionary biology. Before joining BioZone, Katrina worked as a research associate in the Department of Earth Sciences at the University of Toronto specializing in gas chromatography isotope-ratio mass spectrometry. *Katrina is currently filling in for Vinthiya Paramananthasivan who is on maternity leave until Fall 2017.*

Vinthiya Paramananthasivan

Research Grant & Project Administrator

Vinthiya assists with the administration of various grants and projects within Biozone. She holds a Masters's degree in Biological Sciences from the University of Auckland and has previous experience as a Research Administrator at Mount Sinai Hospital and the University of Toronto.

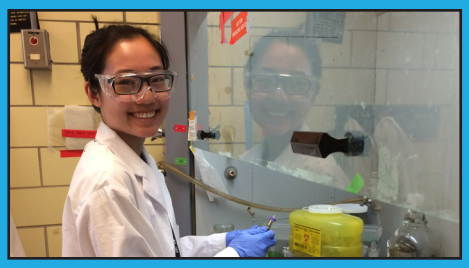
Vinthiya is currently on maternity leave until Fall 2017.

The Undergraduate Experience

An important component of BioZone's mission includes the training and education of undergraduate researchers. Here is what a few of our current undergrads are saying about their time in BioZone:

"Being part of BioZone has definitely been one of the best parts of my undergrad so far. Going into my third summer here, I've learned so much and gained a lot of hands-on laboratory experience. The people I've met are not only intelligent, but also very friendly and helpful. The skills and techniques I've learned in the lab will definitely help me in the future, especially in my fourth year thesis project."

- Amy Li, 4th Year Undergraduate

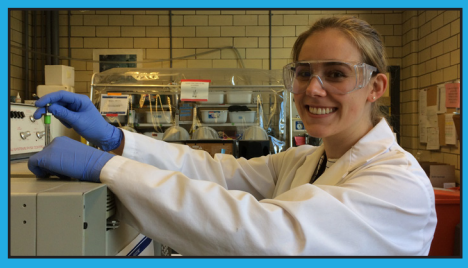


"During my work term I gained a great deal of experience in protein purification and crystallization as well as knowledge regarding the processes of protein crystal formation, improvement and diffraction. Working in the Savchenko lab has been a wonderful learning experience, with fellow lab members going out of their way on more than one occasion to thoroughly explain all aspects of the protein structural determination process to me. Additionally, I found the BioZone community to be not only welcoming and friendly but always willing to discuss their research with me. This was, in itself, a great and enlightening experience, something I have not experienced anywhere else as an undergraduate researcher. The most important thing I will take away from my time in BioZone is the value of open communication between scientific groups with differing goals, which is something I believe the BioZone community has used to its advantage to continually produce the highest quality of scientific research."

- Christopher McChesney, Co-Op Student

"I've been working at BioZone for the past three summers and somehow each one has become more enjoyable. My role here started out as being primarily administrative, but has shifted to involve mostly lab work. This can sometimes be a little intimidating, but I'm always amazed at how easy it is to ask for and receive help, and at how everyone is rooting for their peers to succeed. I think there are few places that foster such an incredible learning experience."

- Rebecca Hough, 4th Year Undergraduate



Alumni Updates

BioZone alumni are involved in exciting endeavours around the world. We are very pleased to be able to include a few updates about their current activities.

Parthiv Amin, *M.A.Sc., 2014*

Currently enrolled in Year 2 of Medical School in the MD Program at the Cumming School of Medicine, University of Calgary.

Nikolaos Anesiadis, *Ph.D., 2014*

I started working at Sanofi-Pasteur, the vaccines division of Sanofi, in 2014 a few months after completing my Ph.D. in Biozone with Professor Mahadevan and Cluett. After working in fermentation/purification for approximately 20 months, I moved to the modeling and data group and used modeling and multivariate analysis to solve high impact problems in the manufacturing and testing of vaccines.

Recently, I decided to move to RBC/Marketing Science group, in which I will use machine learning algorithms to lead business decisions. Big data and machine learning has always been my interest since graduate school and I am very excited for this opportunity. Personally, my wife and I had a son, Maximus, who is 16 months old and we moved in the north part of Richmond Hill.

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Jordan Bouchard, *M.A.Sc., 2015*

I am currently enrolled in a Ph.D. program in the Department of Mechanical and Industrial Engineering at the University of Toronto. My research involves the quenching of porous media beds for the purpose of rapid steam production. Specifically, this is an investigation into the percolation, two-phase flow, and heat transfer of porous media and establishing the effect porous media properties have on the previously mentioned phenomena.

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Thomas Canam, *Postdoctoral Fellow, 2010*

I am currently a tenured Associate Professor of Biological Sciences at Eastern Illinois University (Charleston, IL). My research interests include using molecular tools to explore disease transmission, biochemical pollution, and environmentally-friendly generation of bioenergy and bioproducts.

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Chao Chen, *Postdoctoral Fellow, 2013*

I miss the time that I spent in Biozone, where I received world class scientific training and worked in a very collaborative and friendly environment. Currently, I am working in Dr. James Halpert's lab as a postdoctoral researcher at the School of Pharmacy, the University of Connecticut. My research focus is on function-structure relationship in P450 detoxifying enzymes, and understanding how polymorphism affects drug metabolism and/or ligand binding. My research interests include molecular mechanism of biologically and/or industrially important enzymes as well as exploring their potentials for industrial variability including enzyme discovery, biochemical and biophysical characterization, biocatalysts' multi-variable optimization, biological process development and downstream processing.

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Robyn Goacher, *Postdoctoral Fellow, 2012*

Since leaving Biozone, I have been working as an Assistant Professor of Chemistry at Niagara University in the US. I teach undergraduate courses in analytical, instrumental and environmental chemistry, along with career preparation and communication courses for chemistry and biochemistry students. In recent years, I have had a large research group of ~10 undergraduate students. Our main research areas are studying wood degradation for biofuels and bioproducts, the chemical analysis of wood polymer composites, and study of the deposition order of intersecting inks for forensics. I also continue collaborations with current and former Biozone members.

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Ariel Grostern, *Ph.D., 2009*

After finishing in Elizabeth's lab, I headed to UC Berkeley for a prolonged post-doc working on 1,4-dioxane biodegradation, and that is where my research career ended. After moving to Washington, D.C. in 2013, I joined the American Association for the Advancement of Science in their Research Competitiveness Program, working on projects related to research grant proposal assessment. In 2016, I joined the American Chemical Society, where I am the Managing Editor for the journals Environmental

Science & Technology, Environmental Science & Technology Letters, and Journal of Agricultural and Food Chemistry. My wife Karen (also an UofT alumnus) and I have two kids, Miko (2.5 yo) and Callie (9 months). We are planning to move back up to cooold Canada in the mid- to short-term future, hopefully to a thriving science job market!

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Sam Huang, *M.A.Sc., 2015*

I'm currently working at MetaFLO Technologies Inc. We specialize in liquid waste management by solidifying various types of liquid waste into solid waste. I run a lab performing R&D work as well as doing testing for our clients. I recently got married and will be moving into a house in Milton soon, so real life is really starting.

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Laura Hug, *Ph.D., 2012*

Dr. Laura Hug is currently an Assistant Professor in the Department of Biology at the University of Waterloo. Her research examines microbial diversity and distribution at municipal waste sites, with a focus on identifying novel organisms with bioremediation-relevant activities. Her research group is growing, as is her family, as her son, Gus, arrived in January 2017.

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Tyler Irving, *M.Sc., 2010*

After completing my degree, I became a full-time science writer and communicator. I spent three years as the staff writer for the Canadian Chemical News, a magazine about chemistry and chemical engineering, and earned two national awards for excellence in science journalism. I have done freelance work for a number of publications and outlets, including scripts for AsapSCIENCE, a YouTube channel with more than 5 million subscribers. In 2015, I found my way back to the Faculty and I now produce stories about U of T Engineering research and education for print, web and social media.

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Dragica Jeremic, *Postdoctoral Fellow, 2007*

Currently working as an Assistant Professor in Sustainable Bioproducts Department of Mississippi State University, my area of research is in biodegradation and protection of bioproducts made from wood or other renewable bioresources. Working at BioZone I have gained an invaluable experience in molecular biology techniques related to proteomics and microbial degradation assessment of wood. Above all, I felt that BioZone gave me an opportunity to grow not only as a scientist, but as a lecturer and a professional. Team work and "leading edge" spirit emphasized in the group is something that sets apart BioZone from other research clusters and bring me the most profound memories of pleasure of being once a member of BioZone.

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Lana Kwan, *M.A.Sc., 2012*

For the past two years, I've been working as a Bioprocess Engineering Consultant in the United States. I've been traveling across the east coast working with a variety of biotechnology and pharmaceutical companies in New York, North Carolina, Massachusetts, and Maryland. The work I've done covers qualification, validation, commissioning, quality, and compliance.

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Jacqueline MacDonald, *Ph.D., 2012*

After graduating from BioZone in 2012, I was awarded an NSERC Visiting Fellowship with Agriculture and Agri-Food Canada, where I genetically engineered tobacco for the production of veterinary vaccine candidates. During that time I contributed to four scientific journal articles and edited a book on recombinant protein production in plants. Currently, I am located in London, Ontario, where I teach in the biology department at Western University and the biotechnology degree program at Fanshawe College. I am also employed through Western University's medical school to write and revise scientific publications on microbial biotechnology.

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Farrokh Mansouri, *M.A.Sc., 2014*

I am currently a Ph.D. student at IBBME.

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Nalina Nadarajah, *Research Associate, 2011*

I completed my Ph.D. from Allen lab in 2007. I stayed on as a Research Associate at the Allen lab until May 2011. From June 2011, I have been a full-time professor at the Dept. of Applied Biological and Environmental Science at the School of Engi-

neering Technology and Applied Science at Centennial College. I'm enjoying teaching Environmental Microbiology and Microbial Genetics to students in Advanced Biotechnology program. On personal side, my husband and I welcomed out third son on Christmas day 2015! We enjoy travelling around the world with the kids (so far been to 18 countries)!

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Peter Schnurr, *Ph.D., 2016*

Dr. Peter Schnurr is currently working as a postdoctoral fellow at Ryerson University under the supervision of Michael Arts. Dr. Arts lab specializes in tracking fatty acids through the food chain, with particular emphasis on ecosystem health. Dr. Schnurr is currently working on a number of projects, but his main one is on understanding mudflat algal biofilms on Robert's Bank near Vancouver, B.C. The main focus of this study is to determine temporal and spatial variation of mudflat algal biofilms and the factors that affect their quantity and quality. The results of these studies have potentially serious implications for migratory and shorebird populations in this region, since it one of North America's most significant migratory stopover sights. Other projects Dr. Schnurr is working on in the Arts lab are understanding fatty acid profiles in snapping turtle populations fed various diets and understanding how microplastics in the Great Lakes may affect fish health.

Dr. Schnurr is also a corecipient of a grant called Grand Challenges Canada's Stars in Reproductive, Maternal, Newborn and Child Health. The focus of this grant is to grow *Spirulina* to incorporate into diets of specific demographics of rural Filipino families in order to battle malnutrition.

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Alex Tsai, *Ph.D., 2014*

I have been a postdoctoral fellow in the Joint BioEnergy Institute (JBEI) in Emeryville, California since May 2014 after I've completed my graduate degree in BioZone. My current research focused on discovery and characterizations of enzymes involved in plant cell wall biosynthesis, particularly transferases (ex. acyl-, glycosyl-, acetyl-). I've used molecular biology tools such as *in vitro* characterization, genetic complementation, yeast engineering in conjunction with analytical chemistry techniques including LC/MS, HPLC, and mass spectrometry to elucidate the enzyme's function and provide genetic parts to engineer feedstocks for biofuel/biochemical production.

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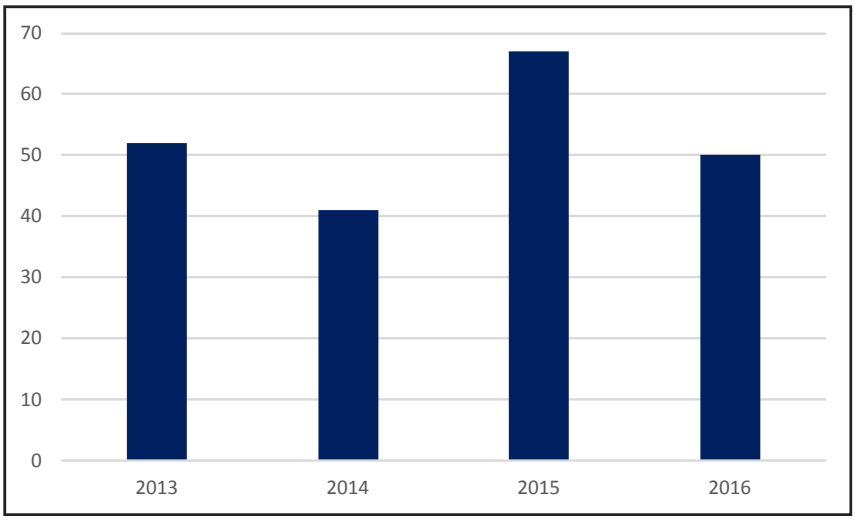
Publications



Number of journal articles and book chapters per year by BioZone research groups

The following publications were published during the September 2015 to August 2016 reporting period.

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Patents

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Loo-Yong-Kee, Steven. (2015). **Bioreactor Scale-up for Protein Production.** M.Eng., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Mansouri, Farrokh. (2015). **Model of Electrophysiology and Metabolism of the Heart.** M.H.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

McRae, Sarah Elizabeth. (2015). **Community Diversity and Functional Capabilities of Benzene-degrading Enrichment Cultures.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Patel, Megha. (2015). **Engineering Enzymes for Carbon Fixing Pathways.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Sarch, Cody. (2015). **Arabinofuranosidase Characterization and Application in Regulating Xylan-Lignin Interactions.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Sherif, Mohammed. (2015). **Using Esterase and Laccase Enzymes to Derivatize Bioactive Plant Phenolics for Altered Chemistry.** Ph.D., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Singh, Krista Nicole. (2015). **The Effect of Orifice Flow Treatment on Biosludge Dewaterability.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Tomory, John. (2015). **Biosludge Dewatering using Freeze/Thaw.** M.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Wang, Fanming (Shirley). (2015). **Stability of Salt Double-Fortified with Iodine and Iron.** M.Eng., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Yang, Minqing. (2015). **The Effect of Anaerobic Treatment of Pulp Mill Effluents on Reactor Performance and Granular Sludge.** Ph.D., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Zhou, Han (Wendy). (2014). **Nitrate-Dependent, pH Neutral Bioleaching of Ni from an Ultramafic Concentrate.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Conference Invited Talks

Chen, X., Howe, Y.J., Woo, P., Perovic, D., Edwards, E.A. **Application of ionic liquid on biological samples in correlative optical microscopy and scanning electron microscopy,** invited talk at the *Microscopy & Microanalysis Conference*. Columbus, Ohio. July 26, 2016.

Edwards, E. **Anaerobic digestion and other water technologies,** invited talk at the *Industrial Workshop on Water Innovation*. Toronto, ON. June 17, 2016.

Edwards, E. **Anaerobic microbial consortia: An enriching experience that started for me at Stanford,** invited talk at the *Perry McCarty Distinguished Lecture*. Stanford, CA. April 29, 2016.

Edwards, E. **Cultivating dark matter,** invited talk at the *International Society for Microbial Ecology Conference*. Montreal, QC. August 21-26, 2016.

Mahadevan, R. **Genome-based modeling of microbial communities relevant to bioremediation,** invited talk at the *EMBL Heidelberg - The European Molecular Biology Laboratory*. Heidelberg, Germany. June 2016.

Mahadevan, R. **Orthogonal pathway design for metabolic engineering,** invited talk at the *Max Planck Institute of Dynamics of Complex Systems*. Magdeburg, Germany. June 2016.

Mahadevan, R. **Role of redundancy and synthetic rescues in metabolic engineering,** invited talk at the *Network Frontier Workshop*. Evanston, Illinois. December 6-7, 2015.

Master, E.R. **Carbohydrate active enzymes for new hemicellulose-derived polymers,** invited talk at the *Symposium on Lignin and Hemicellulose Valorization*. Lund, Sweden. November 4, 2015.

Master, E.R. **Carbohydrate oxidases for new hemicellulose-derived polymers. Refining Lignocellulosics to Advanced Polymers and Fibers,** invited talk at the *PolyRefNorth*. Ås, Norway. November 26-27, 2015.

Master, E.R. **Characterizing lignocellulolytic enzymes through surface analysis techniques,** invited talk at the *4th Symposium on Biotechnology applied to Lignocelluloses (Lignobiotech IV)*. Madrid, Spain. June 19-22, 2016.

Master, E.R. **New approaches to uncovering function from fungal genomes,** invited talk at the *13th European Conference on Fungal Genetics*. Paris, France. April 3-6, 2016.

McGuigan, A. **Designing predictive *in vitro* tissue mimetic systems for drug discovery,** invited talk at the *Tufts University*. Boston, Massachusetts. February 18, 2016.

McGuigan, A. **TRACER: A 3D engineered tumor for quantifying spatial metabolic reprogramming in hypoxic gradients,** invited talk at the *Functional Analysis and Screening Technologies Congress*. Boston, Massachusetts. November 10-11, 2015.

McGuigan, A. **TRACER: An engineered tumour for exploring cellular phenotype and microenvironment in hypoxic gradients,** invited talk at the *Cancer Research UK Cambridge Institute Annual International Symposium*. Cambridge, UK. March 4-5, 2016.

Savchenko, A. **Getting in on the act: Prokaryotic pathogens meddle with host ubiquitination,** invited talk at the *Canadian Society of Microbiologists Annual Conference*. Toronto, ON. June 12-15, 2016.

Savchenko, A. **Getting in on the act: Prokaryotic pathogens meddle with host ubiquitination,** invited talk at the *Keystone Symposia: Ubiquitin Signalling (X3)*. Whistler, BC. March 13-17, 2016.

Savchenko, A. **Getting in on the act: Prokaryotic pathogens meddle with host ubiquitination,** invited talk at the *Banff Conference on Infectious Disease*. Banff, AB. June 1-4, 2016.

Savchenko, A. **Structural and functional characterization of the environmental antibiotic resistome,** invited talk at the *Banff Conference on Infectious Diseases*. Banff, AB. June 1-4, 2016.

Savchenko, A. **Structural and functional characterization of the environmental antibiotic resistome,** invited talk at the *Canadian Society of Microbiologists Meeting*. Toronto, ON. June 12-15, 2016.

Savchenko, A. **Structural characterization of aminoglycoside antibiotic resistance enzymes from environmental and human microbiomes,** invited talk at the *CSGID/SSGCID Annual Programmatic Meeting*. Seattle, Washington. May 17, 2016.

Conference Oral Presentations

Boëns, B., Bourdeau, N., Gélinas, M., Pilon, G., Tremblay, C., Obnamia, J.A., MacLean, H.L., Saville, B.A., Adjallé, K., Barnabé, S. **Sustainable algal biorefineries? A journey through the RTA colocation project,** oral presentation at the *BIO World Congress on Industrial Biotechnology*. San Diego, California. April 17-20, 2016.

Correia, K., Li, P., Yu, L., Mahadevan, R. **Reconstruction of a yeast pan-genome-scale metabolic model for evolutionary systems biology,** oral presentation at the *17th European Congress on Biotechnology*. Krakow, Poland. July 3-6, 2016.

D'Arcangelo, E., A.P., M. **Engineering organotypic tumor microenvironment models,** oral presentation at the *International Society of Cancer Metabolism Annual Meeting*. Venice, Italy. September 16-19, 2015.

Diosady, L.L. **Food engineering - Chemical engineers in the worlds largest industry,** oral presentation at the *BioZone Symposium*. Toronto, ON. November 13, 2015.

Dueik, V.P., Diosady, L. **Iron fortification of tea for prevention of maternal mortality in South Asia,** oral presentation at the *International Union of Food Science and Technology - 18th World Congress on Food Science and Technology*. Dublin, Ireland. August 24, 2016.

Euler, C.E., Mahadevan, K. **The need for speed (and precision): Protein-level control in a model system,** oral presentation at the *Quebec-Ontario Biotechnology Meeting*. Waterloo, ON. May 26-27, 2016.

Genin, S.N., Aitchison, J.S., Allen, D.G. **Novel waveguide for enhancing algal biofilm growth,** oral presentation at the *65th Canadian Chemical Engineering Conference*. Calgary, AB. October 16-19, 2015.

Guilford, N., Edwards, E. **Comparative life cycle inventory analysis of alternative methods for organic solid waste management** oral presentation at the *A&WMA/ONEIA Conference*. October 7, 2015.

Klamt, S., Mahadevan, R. **Feasibility of growth-coupled product synthesis in microbial strains,** oral presentation at the *4th Conference on Constraint-Based Reconstruction and Analysis*. Heidelberg, Germany. September 16-18, 2015.

Krishnaswamy, K., Diosady, L. **Food fortification: An effective approach to improve global health,** oral presentation at the *Engineering World Health Symposium*. Toronto, ON. February 22, 2016.

Li, A., Lomheim, L., Edwards, E.A. **Composition of the microbial community in microcosms degrading chlorinated aromatic compounds,** oral presentation at the *Undergraduate Engineering Research Day*. Toronto, ON. August 9, 2016.

Li, Y.O., Diosady, L.L. **Production of rice fortified with reconstituted, extruded rice grains stabilized by internal gelation of Ca-alginate,** oral presentation at the *IUFoST 18th World Congress on Food Science and Technology*. Dublin, Ireland. August 24, 2016.

Lu, S., Mahadevan, R. **Pathway prioritization using protein cost,** oral presentation at the *CSCHE Quebec-Ontario Biotechnology Meeting*. Waterloo, ON. May 26-27, 2016.

Lu, S., Mahadevan, R. **Pathway prioritization using protein cost,** oral presentation at the *Industrial Biocatalysis Network (IBN) Annual General Meeting*. Montreal, QC. May 18, 2016.

MacCormick, B., Master, E.R. **Adding value to underrepresented biomass fractions: Pathways to hemicellulose-based polymer synthesis,** oral presentation at the *Annual General Meeting of the Industrial Biocatalysts Network* Montreal, QC. May 18-19, 2016.

MacCormick, B., Master, E.R. **Applications of gluco-oligosaccharide oxidases for carbohydrate modification,** oral presentation at the *5th International Conference on Biorefinery and Bioenergy*. Vancouver, BC. August 10-12, 2016.

Mahadevan, R., von Kamp, A., Klamt, S. **Strain design with constrained regulatory minimal cut sets in genome-scale metabolic networks,** oral presentation at the *4th Conference on Constraint-Based Reconstruction and Analysis*. Heidelberg, Germany. September 16-18, 2015.

Malekzai, R., Master, E.R. **Hydrophobin coatings for improved water barrier properties of cellulose packaging,** oral presentation at the *Ontario-Quebec Biotechnology Meeting*. Waterloo, ON. May 26, 2016.

McGee, E., Diosady, L. **Fortification of tea with iron,** oral presentation at the *International Conference on Food Chemistry and Hydrocolloids*. Toronto, ON. August 11, 2016.

McGee, E., Diosady, L. **Fortification of tea with iron,** oral presentation at the *International Conference on Food Chemistry & Technology*. San Francisco, California. November 17, 2015.

McGee, E., Diosady, L. **Model system points to strategies for the fortification of tea with iron,** oral presentation at the *International Conference on Food Chemistry and Technology*. San Francisco, California. November 17, 2015.

Meyer, T., Edwards, E.A., Tran, H., Allen, D.G. **Handling and dewatering of pulp and paper mill biosludge,** oral presentation at the *Paper Week Canada Meeting*. Montreal, QC. February 1-4, 2016.

Nouraei, M., Acosta, E., Diosady, L.L. **Microemulsions as extracting and delivery systems for nutraceuticals,** oral presentation at the *PMAFRA Product Development Research Day*. Guelph, ON. March 7, 2016.

Obnamia, J.A., MacLean, H.L., Saville, B.A. **Comparative evaluation of LCA models on biofuels: A case study on corn stover ethanol,** oral presentation at the *65th Canadian Chemical Engineering Conference*. Calgary, AB. October 4-7, 2015.

Quaile, A.T., Palys, S., Miotto, L.S., DeFalco, M., Tsang, A., Savchenko, A. **Overcoming the challenges of activating secondary metabolite gene clusters in *Aspergillus niger*,** oral presentation at the *Industrial Biocatalysis Network: Annual General Meeting*. Montreal, QC. May 18-19, 2016.

Rodenhizer, D., Cojocari, D., Gaude, E., Mahadevan, K., Wouters, B., Frezza, C., McGuigan, A.P. **TRACER: A 3D engineered tumour for quantifying spatial metabolic reprogramming in hypoxic gradients,** oral presentation at the *American Society for Mass Spectrometry Meeting*. San Antonio, Texas. June 5-9, 2016.

Schnurr, P.J., Espie, G.S., Allen, D.G. **Factors affecting algae biofilm growth kinetics and internal lipid concentrations: Light and nutrients,** oral presentation at the *2015 Algal Biomass Summit*. Washington D.C. September 29 - October 2, 2015.

Soleas, J., McGuigan, A. **Geometric manipulation of lung progenitors to control cell fate choice,** oral presentation at the *World Biomaterials Conference*. Montreal, QC. May 17-22, 2016.

Soleas, J.P., A.P., M., Waddell, T.K. **Architechtural Manipulation of multipotent lung progenitors to control cell fate choice,** oral presentation at the *World Biomaterials Conference*. Montreal, QC. May 17-22, 2016.

Stogios, P., Savchenko, A. **Structural and functional characterization of the environmental antibiotic resistome,** oral presentation at the *Banff Conference on Infectious Diseases*. Calgary, AB. June 1-5, 2016.

Stogios, P., Savchenko, A. **Structural characterization of aminoglycoside antibiotic resistance enzymes from environmental and human microbiomes,** oral presentation at the *CSGID/SSGCID Annual Programmatic Meeting*. Seattle, Washington. May 17-18, 2016.

Venkatesan, K., Partow, S., Mahadevan, R. **Towards the complete biosynthesis of adipic acid in *Saccharomyces cerevisiae*,** oral presentation at the *CSChE Quebec-Ontario Biotechnology Meeting*. Waterloo, ON. May 26-27, 2016.

Venkatesan, K., Partow, S., Mahadevan, R. **Towards the complete biosynthesis of adipic acid,** oral presentation at the *Industrial Biocatalysis Network Annual General Meeting*. Montreal, QC. May 2016.

Wang, P.S., Tang, S., Nemr, K., Flick, R., Edwards, E.A. **Two isolates capable of organohalide respiration with chloroform or chlorinated C2 alkanes reveal novel metabolic features of the *Dehalobacter* genus,** oral presentation at the *International Society for Microbial Ecology*. Montreal, QC. August 25, 2016.

Wang, W., Master, E. **Enzymes for biopolymer synthesis and recovery from plant resources,** oral presentation at the *Ontario-China Biomaterial Workshop*. Toronto, ON. December 7, 2015.

Wang, W., Master, E.R. **Enzymes for biopolymer synthesis and recovery from plant resources,** oral presentation at the *Ontario-China Biomaterial Workshop*. Toronto, ON. December 7, 2015.

Wong, M.T., Couturier, M., Razeq, F.M., Wang, W.J., Terrapon, N., Lombard, V., Henrissat, B., Edwards, E.A., Master, E.R. **Metagenomic analysis of microbial consortia from moose and beaver digestive systems following long-term enrichment on cellulosic substrates** oral presentation at the *NSERC Industrial Biocatalysis Network Annual General Meeting*. Montreal, QC. May 2016.

Xianjin, T., Puentes Jacome, L.A., Qiao, W., Yang, M., Avanzi, I.R., Lomheim, L., Edwards, E.A. **Comparison of microbial communities degrading hexachlorocyclohexane isomers in enrichment cultures through pyrosequencing of DNA and cDNA,** oral presentation at the *International Society for Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Yang, M.I., Edwards, E.A., Allen, D.G. **Using amplicon sequencing to investigate the impact of high-strength pulp mill effluent on the microbial communities of anaerobic sludge,** oral presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Conference Poster Presentations

Choolaei, Z., Bonilla, S., Yakunin, A.F., Allen, D.G., Edwards, E.A. **Enzymatic pretreatment of pulp and paper mill biosludge for enhancing its anaerobic digestibility,** poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Choolaei, Z., Yakunin, A. **Enzymatic pretreatment of pulp and paper mill biosludge for enhancing its anaerobic digestibility,** poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Euler, C., Mahadevan, R. **Protein-level control of metabolism: Design principles and prospects from a representative system,** poster presentation at the *Industrial Biocatalysis Network AGM*. Montreal, QC. May 18-19, 2016.

Gaona, A., Lawryshyn, Y., Saville, B. **Evaluating the mixing performance of high-solids lignocellulosic saccharification through experimental and CFD approaches,** poster presentation at the *Mixing XXV Conference*. Quebec City, QC. June 26-July 1, 2016.

Guilford, N., Edwards, E. **Design, construction and commissioning of a laboratory-scale anaerobic digester for organic solid waste, comprising sequentially-fed leach beds coupled to an upflow anaerobic sludge blanket, AD14** poster presentation at the *Anaerobic Digestion Symposium*. November 2015.

Hajighasemi, M., Nocek, B., Popovic, A., Tchigvintsev, A., Brown, G., Flick, R., Zhu, X., Xu, X., Cui, H., Joachimiak, A., Savchenko, A., Edwards, E., Yakunin, A. **Enzymatic depolymerization of biodegradable polyesters by microbial carboxylesterases,** poster presentation at the *Gordon Research Conference on Biocatalysis*. Biddeford, Maine. July 10-15, 2016.

Hajighasemi, M., Nocek, B., Tchigvintsev, A., Brown, G., Flick, R., Xu, X., Cui, H., Joachimiak, A., Savchenko, A., Edwards, E.A., Yakunin, A.F. **Enzymatic depolymerization of polylactic acid by two microbial carboxylesterases,** poster presentation at the *CSM 66th Annual Conference*. Toronto, ON. June 12-15, 2016.

Hajighasemi, M., Nocek, B., Zhu, X., Tchigvintsev, A., Brown, G., Flick, R., Xu, X., Cui, H., Joachimiak, A., Savchenko, A., Edwards, E., Yakunin, A.F. **Enzymatic depolymerization of biodegradable polyesters by microbial carboxylesterases,** poster presentation at the *Biocatalysis Gordon Research Conference*. Biddeford, Maine. July 9-15, 2016.

Hamemeh, R., Loo-Yong-Kee, S., Bonilla, S., Allen, D.G. **Scaling-Up protein production on *Escherichia coli*: Effect of induction temperature profile on cell yield in a pilot-scale fermentation unit,** poster presentation at the *18th Canadian Society for Chemical Engineering Ontario-Quebec Biotechnology Meeting*. Waterloo, ON. May 26, 2016.

Khusnutdinova, A., Flick, R., Brown, G., Joo, J.C., Mahadevan, R., Yakunin, A.F. **New microbial enzymes for the biosynthesis of adipic acid and other commodity chemicals,** poster presentation at the *66th Annual Conference of Canadian Society of Microbiologists*. Toronto, ON. June 12-15, 2016.

Khusnutdinova, A.N., Flick, R., Brown, G., Joo, J.C., Mahadevan, R., Yakunin, A.F. **New microbial enzymes for the biosynthesis adipic acid and other commodity chemicals,** poster presentation at the *IBN Annual General Meeting*. Montreal, QC. May 18-19, 2016.

Khusnutdinova, A.N., Flick, R., Brown, G., Joo, J.C., Mahadevan, R., Yakunin, A.F. **New microbial enzymes for the biosynthesis adipic acid and other commodity chemicals,** poster presentation at the *Autotrophic Microorganisms: 5th All-Russian Symposium*. Moscow, Russia. December 21-24, 2015.

Kraus, S., Edwards, E.A. **Evaluation of potential inhibitory effects in complex contaminant mixtures,** poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Kwan, R., Vuong, T.V., Foumani, M., MacCormick, B., Master, E.R. **Increasing oligosaccharide oxidase (AA7) stability and substrate range through protein engineering and discovery,** poster presentation at the *Annual General Meeting of the Industrial Biotechnology Network*. Montreal, QC. May 18-19, 2016.

Lee, H., Guilford, N.G.H., Edwards, E.A. **Characterization of the microbial community of an anaerobic digester treating solid waste,** poster presentation at the *International Society for Microbial Ecology*. Montreal, QC. August 25, 2016.

Lemak, S., Kuznedelov, K., Semenova, E., Datsenko, K.A., Severinov, K., Yakunin, A.F. **Characterization and engineering of the RNA-guided cascade complex from *Escherichia coli*,** poster presentation at the *66th Annual Conference of CSM*. Toronto, ON. June 12-15, 2016.

Luo, F., McRae, S.E., Edwards, E.A. **Electron acceptor-driven microbial community shifts in the anaerobic benzene-degrading cultures,** poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

MacCormick, B., Master, E. **Bringing value-added products and services to overlooked lignocellulosic fractions,** poster presentation at the *BioZone Horizons Symposium*. Toronto, ON. August 12, 2016.

Magnuson, E., Luo, F., Edwards, E.A. **Microbial community dynamics in nitrate-reducing benzene-degrading cultures,** poster presentation at the *International Society for Microbial Ecology Conference*. Montreal, QC. August 25-26, 2016.

Meyer, T., Chen, L., Huang, X.M., Edwards, E. **Anaerobic digestion of pulp and paper mill wastewater and sludge,** poster presentation at the *14th World Congress on Anaerobic Digestion*. Vina del Mar, Chile. November 14-18, 2015.

Molenda, O., Edwards, E.A. **Vinyl chloride reductase (vcrA) containing genomic island mobilizes in *Dehalococcoides* in the KB-1 mixed microbial consortium used for bioremediation,** poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Mollerup, F., Parikka, K., Tenkanen, M., Master, E. **Engineering new family AA5 galactose oxidases,** poster presentation at the *OxiZymes Meeting*. Wageningen, Netherlands. July 3-6, 2016.

Muhammad Razeq, F., Master, E. **Production and characterization of a protein with unknown function from polysaccharide utilization loci encoding xylan-active enzymes,** poster presentation at the *NSERC Industrial Biocatalysis Network General Meeting*. Montreal, QC. May 18-19, 2016.

Nemr, K., Mahadevan, R. **Engineering a short, aldolase-based pathway for 1,3-butanediol production in *Escherichia coli*,** poster presentation at the *Metabolic Engineering 11 Meeting*. Awaji, Japan. June 26-30, 2016.

Pandit, A.V., Mahadevan, R. **Orthogonal Design of Metabolic Pathways,** poster presentation at the *Metabolic Engineering Conference – XI*. Kobe, Japan. June 26-30, 2016.

Partow, S., Hyland, P., Mahadevan, R. **Synthetic rescue couples NADPH generation to metabolite overproduction in *Saccharomyces cerevisiae*,** poster presentation at the *XI Metabolic Engineering Conference*. Kobe, Japan. June 26-30, 2016.

Poon, J.C.H., Nguyen, Q., Soleas, J.P., Liao, Z., Aitchison, J.S., Mennella, V., McGuigan, A.P., Waddell, T.K. **Controlling the microenvironment of human airway epithelial cells for tracheal tissue engineering,** poster presentation at the *10th World Biomaterials Congress*. Montreal, QC. May 17-22, 2016.

Puentes Jacome, L.A., Edwards, E.A. **Reductive dechlorination of 1,2,4 trichlorobenzene by the *Geobacter lovleyi* sp. in the KB-1 bioaugmentation culture,** poster presentation at the *4th INTEGRATE Annual General Meeting*. London, ON. October 1-2, 2015.

Puentes Jacome, L.A., Wang, P., Lomheim, L., Edwards, E.A. **Microbial community changes in response to 1,2,4-trichlorobenzene dechlorination based on 16S amplicon pyrosequencing of DNA and cDNA in KB-1, a mixed microbial culture used for bioremediation,** poster presentation at the *International Society for Microbial Ecology Conference*. Montreal, QC. August 21-26, 2016.

Quaile, A.T., Egorova, O., Stogios, P.J., Evdokimova, E., Nocek, B., Cuff, M., Kompella, P., Peisajovich, S., Ensminger, A.W., Yakunin, A.F., Savchenko, A. **Modulation of eukaryotic MAPK pathways by a *Legionella pneumophila* Dot/Icm effector with phosphotyrosine phosphatase activity,** poster presentation at the *Canadian Society of Microbiologists Annual Conference*. Toronto, ON. June 12-15, 2016.

Razeq, F.M., Master, E.R. **Production and characterization of a protein with unknown function from polysaccharide utilization loci encoding xylan-active enzymes,** poster presentation at the *NSERC Industrial Biocatalysis Network Meeting*. Montreal, QC. May 2016.

Rodenhizer, D., Cojocari, D., Gaude, E., Mahadevan, K., Wouters, B., Frezza, C., McGuigan, A.P. **TRACER: A 3D engineered tumour for mapping cell metabolism and phenotype in heterogeneous microenvironments,** poster presentation at the *World Biomaterials Congress*. Montreal, QC. May 17-22, 2016.

Rodenhizer, D., Cojocari, D., Gaude, E., Mahadevan, K., Wouters, B.G., Frezza, C., McGuigan, A.P. **TRACER: Tissue roll for analysis of cellular environment and response,** poster presentation at the *IBBME Scientific Day*. Toronto, ON. May 5, 2016.

Soleas, J., McGuigan, A. **Geometric manipulation of multipotent lung epithelial progenitors to control cell fate choice,** poster presentation at the *Stem Cells, Cellular Therapies, and Bioengineering in Lung Biology and Lung Diseases Conference*. Burlington, Vermont. July 27-30, 2016.

Stogios, P., Savchenko, A. **Structural and functional characterization of the environmental antibiotic resistome,** poster presentation at the *Canadian Society of Microbiologists Meeting*. Toronto, ON. June 13-16, 2016.

Xiao, J., Luo, F., Khusnutdinova, A., Wang, P., Edwards, E. **Using *Clostridium* to heterologously express enzymes from strict anaerobes: The case of anaerobic benzene carboxylation,** poster presentation at the *Industrial Biocatalysis Network Annual General Meeting*. Montreal, QC. May 18-19, 2016.

Xiao, J., Luo, F., Khusnutdinova, A., Wang, P., Edwards, E. **Using *Clostridium acetobutylicum* to heterologously express enzymes from obligate anaerobes: the case of putative anaerobic benzene carboxylase,** poster presentation at the *International Symposium on Microbial Ecology Conference*. Montreal, QC. August 21-26, 2016.

Xiao, J., Luo, F., Khusnutdinova, A., Wang, P., Edwards, E.A. **Using *Clostridium acetobutylicum* to heterologously express enzymes from obligate anaerobes: The case of putative anaerobic benzene carboxylase,** poster presentation at the *Clostridium XIV Conference*. Hanover, New Hampshire. August 28-31, 2016.

Ya, J., Tran, H., Allen, D.G. **Electro-dewatering of biosludge,** poster presentation at the *Paper Week Canada Meeting*. Montreal, QC. February 1-4, 2016.

Grants, Awards & Scholarships

During 2015 and 2016, BioZone researchers, postdoctoral fellows and students were recognized for their ongoing excellence in research, teaching and communication with scholarships, awards and professional accolades. Several of our professors also received prestigious grants from Canadian and international funders in support of their innovative research programs.

Grants

The following grants were operational during the period between September 2015 to August 2016.

International

BHIVE (Bio-derived HIgh Value polymers through novel Enzyme function)
Sponsor: European Research Council
PI: Emma Master

Center for Structural Genomics of Infectious Diseases
Sponsor: National Institutes of Health Subgrant (Northwestern University)
PI: Alexei Savchenko

Collaborative Research: Functional and Evolutionary Bases of Substrate-Specificity in Wood-Decaying Basidiomycetes
Sponsor: National Science Foundation Subgrant (Clark University)
PI: Emma Master

Exploitation of Host Ubiquitination Pathways by Pathogenic E. coli
Sponsor: Canadian Institutes of Health Research
PI: Alexei Savchenko

Canadian

A Novel 3D Tissue-Engineered Platform to Identify Novel Therapy Targets in Head and Neck Squamous Cell Carcinoma
Sponsor: Canadian Institutes of Health Research
PI: Alison McGuigan

A Tissue Engineering Platform to Identify Target Combinations in Tumour Stoma to Render Tumours Therapy Sensitive
Sponsors: Canadian Institutes of Health Research - Collaborative Health Research Project, Natural Sciences and Engineering Research Council of Canada - Collaborative Health Research Project Grant
PI: Alison McGuigan

Anaerobic Digestion of Solid Waste: A New Approach to Accommodating Feedstock Variability to Achieve Stable Operation at an Affordable Cost
Sponsor: Natural Sciences and Engineering Research Council of Canada – Collaborative Research and Development Grant
PI: Elizabeth Edwards

Banting Postdoctoral Fellowship Awards
Sponsor: Canadian Institutes of Health Research - Banting Postdoctoral Fellowship
PI: Levente Diosady

Bioleaching of Nickeliferous Pyrrhotite Tailings: Bioprocessing of Nickeliferous Sulphidic Wastes from Mining Activities
Sponsor: Natural Sciences and Engineering Research Council of Canada – Collaborative Research and Development Grant
PI: Vladamiros Papangelakis
Co-Investigators: Elizabeth Edwards, Radhakrisnan Mahadevan

Biological Production of Xylitol from Hydrolyzates
Sponsors: Connect Canada, Ontario Centres of Excellence
PI: Bradley Saville

Brassica Oilseed Protein Processing
Sponsor: Natural Sciences and Engineering Research Council of Canada - Idea to Innovation Grant
PI: Levente Diosady

Canada Research Chair in Anaerobic Biotechnology
Sponsor: Natural Sciences and Engineering Research Council of Canada - Canada Research Chair
PI: Elizabeth Edwards

CFD Modelling of Biomass Hydrolysis
Sponsor: Natural Sciences and Engineering Research Council of Canada – Discovery Grant
PI: Bradley Saville

Combined Enzymatic and Mechanical Processing of Spent Ethanol Yeast for Recovery of High-Value Bioproducts
Sponsor: Natural Sciences and Engineering Research Council of Canada – Engage Grant
PI: Emma Master

Dean Strategic Fund: MS Facility (DSF14-17)
Sponsor: University of Toronto; Faculty of Applied Science & Engineering – Dean Strategic Fund

Developing Biofilm Microalgal Bioreactors for Efficient Production of Fuels, Chemicals and Clean Water
Sponsor: Natural Sciences and Engineering Research Council of Canada – Strategic Partnership Grant
PI: D. Grant Allen

Development of 100% Biobased Adhesive for Applications in Engineered Wood Products
Sponsors: Ecosynthetix, Natural Sciences and Engineering Research Council of Canada – Engage Grant
PI: Emma Master

Enhanced Anaerobic Bioremediation of Chlorinated Pesticides and their Metabolites in Soil and Groundwater: Development and Demonstration of Field-Ready Technologies
Sponsor: Ontario Ministry of Research, Innovation and Science - Ontario-China Research and Innovation Fund
PI: Elizabeth Edwards

Enhancing Productivity and Cognitive Ability of 10 Million Rural Agricultural People in Uttar Pradesh in India via *Scale-Up and Consumption of Double Fortified Salt*
Sponsor: International Development Research Centre
PI: Levente Diosady

Environmental and Economic Analysis on a Life Cycle Basis of Canadian H2 Sources for Biofuel Production
Sponsor: Networks of Centres of Excellence: BioFuelNet
PI: Bradley Saville

Industrial Biocatalysis Network
Sponsors: Natural Sciences and Engineering Research Council of Canada – Strategic Partnership Grant, CanSyn Chemical Corporation, E.I. DuPont Canada, ELANCO - Animal Health: A Division of Eli Lilly Canada, Lallemand, Monaghan Biosciences, SunCor, BP Technology Ventures
PI(s): Elizabeth Edwards, Radhakrishnan Mahadevan, Emma Master, Alexei Savchenko, Alexander Yakunin

Inhibition of Human Nucleotide Sanitation Enzymes for Cancer Suppression
Sponsor: Canadian Cancer Society Research Institute
PI: Alexander Yakunin

Investigating the Development of 100% Biobased Adhesive for Applications in Engineered Wood Products
Sponsor: Natural Sciences and Engineering Research Council of Canada – Engage Grant
PI: Emma Master

Market Assessment Study for Bio-based 1,3-Butanediol as a Specialty Chemical in North America
Sponsor: Natural Sciences and Engineering Research Council of Canada - Idea to Innovation Grant
PI: Radhakrishnan Mahadevan

Metagenomic Study of Dechlorinating Microbial Communities in vitro and in situ
Sponsor: MITACS - Accelerate Ontario
PI: Elizabeth Edwards

Ontario-China Young Scientist Exchange Program (YSEP)
Sponsor: Ontario Ministry of Research, Innovation and Science - Ontario-China Young Scientist Exchange
PI: Elizabeth Edwards

Pathway Search Using Simulated Annealing
Sponsor: Natural Sciences and Engineering Research Council of Canada - U of T Excellence Award
PI: Radhakrishnan Mahadevan

Production of Bio-based 1,3-butanediol
Sponsor: Connaught Fund - Innovation Award
PI: Radhakrishnan Mahadevan

Quadruple Fortified Salt: An Efficient and Scaleable Vehicle for Simultaneous Delivery of Iron, Folic Acid, Vitamin B12 and Iodine in Low Resource Settings
Sponsor: Grand Challenges Canada - Saving Lives at Birth
PI: Levente Diosady

Reducing Maternal Mortality by Improving Iron Status of Women through Iron Fortification of Tea
Sponsor: Grand Challenges Canada - Saving Lives at Birth
PI: Levente Diosady

Remediation Education Network (RENEW) Training Program
Sponsor: Natural Sciences and Engineering Research Council of Canada – Collaborative Research and Training Experience Program
PI: Brent Sleep
Co-Investigator: Elizabeth Edwards

Solving the Antibiotic Resistance Crisis
Sponsor: Ontario Research Fund – Research Excellence Subgrant (McMaster University)
PI: Alexei Savchenko

Strategic Network Enhancement Initiative
Sponsor: Natural Sciences and Engineering Research Council of Canada – Strategic Partnership Grant
PI: Elizabeth Edwards

Synbiomics: Functional genomics and technoeconomic models for advanced biopolymer synthesis
Sponsor: Genome Canada – Large-scale Applied Research Project
PIs: Emma Master and Elizabeth Edwards

Awards

International

Biochemical Engineering Journal - Outstanding Reviewer Award, 2015
Elsevier
Thu Vuong

Lifetime Achievement Award, 2015
American Oil Chemists' Society
Levente Diosady

Lifetime Achievement Award, 2015
International Association of Engineering and Food
Levente Diosady

Order of Merit of the Republic of Hungary, Officers' Cross, 2015
Levente Diosady

Canadian

BioFuelNet HQP Rapid Fire Presentation, 2015
BioFuelNet Symposium, Montreal
(1st Place)

BioFuelNet HQP Travel Award, 2015
BioFuelNet Symposium, Montreal

Canada Research Chair (Tier 1) in Anaerobic Biotechnology, 2014 to present
Government of Canada
Elizabeth Edwards

Erwin Edward Hart Professorship, 2016
Faculty of Applied Science & Engineering
Alison McGuigan

Fellow of the Royal Society of Canada, 2015
Levente Diosady

Graduate Oral Poster Presentation, 2015
Protein Structure, Function and Malfunction Meeting, University of Saskatchewan, Canada
(1st Place)

Killam Prize, 2016
Canada Council for the Arts
Elizabeth Edwards

Student Discovery Award, Multiple Years
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Student Paper Award, 2015
GeoSyntec Consultants

U of T Conference Grant, Multiple Years
University of Toronto; School of Graduate Studies

Student Scholarships

Applied Science Graduate Faculty Fellowship
University of Toronto

CIHR Training Program in Regenerative Medicine Graduate Fellowship Award
Canadian Institutes of Health Research

Departmental Fellowship
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Diran Basmadjian Graduate Scholarship in Chemical Engineering & Applied Chemistry
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Doctoral Completion Award
University of Toronto; School of Graduate Studies

Dr. Goran Enhorning Award in Pulmonary Research
University of Toronto; Faculty of Medicine

Dr. Joe A. Connolly Memorial Award
University of Toronto; Faculty of Medicine

Eco-Tec Founder's Scholarship
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Eric David Baker Krause Graduate Fellowship
University of Toronto; School of the Environment

Frank Howard Guest Bursary (2)
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Helen L. Cross Memorial Graduate Scholarship
University of Toronto; Department of Chemical Engineering & Applied Chemistry

McLaughlin Foundation Award for MD/PhD Students
University of Toronto; Faculty of Medicine

McLean Foundation Graduate Scholarship in Science and Technology
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Mitacs Accelerate Fellowship
MITACS

Mitacs Globalink Graduate Fellowship
MITACS

NSERC Canada Graduate Scholarships - Master's Program (4)
Natural Sciences and Engineering Research Council of Canada

NSERC CREATE in Manufacturing, Materials and Mimetics (M3) (3)
Natural Sciences and Engineering Research Council of Canada

NSERC Postgraduate Scholarships-Doctoral Program (5)
Natural Sciences and Engineering Research Council of Canada

NSERC Undergraduate Student Research Awards
Natural Sciences and Engineering Research Council of Canada

Ontario Graduate Scholarship (9)
Ontario Ministry of Training, Colleges and Universities

Ontario Trillium Scholarship
Ontario Ministry of Training, Colleges and Universities

Paul Cadario Doctoral Fellowship in Global Engineering
University of Toronto; Faculty of Applied Science & Engineering

Professor William F. Graydon Memorial Graduate Fellowship
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Queen Elizabeth II Graduate Scholarship in Science & Technology
Ontario Ministry of Training, Colleges and Universities

State Scholarship Fund
China Scholarship Council

T-Holder's Academic Excellence Awards
University of Toronto; Varsity Blues

University of Toronto Fellowship
University of Toronto

W.H. Rapson Memorial Award
University of Toronto; Department of Chemical Engineering & Applied Chemistry

William and Dorothy Palm Graduate Scholarship in Science and Technology
Women's College Research Institute

William Dowkes Graduate Bursary
University of Toronto; Department of Chemical Engineering & Applied Chemistry

Outreach & Events

BioZone students, personnel and PIs organize and participate in a large variety of research-related events, volunteer activities and social outings. In addition to regular meetings among individual research groups, the events listed below highlight some of the activities that BioZone has taken part in to foster the exchange of ideas.

Research & Training

BioZone Research Symposium and BioZone Horizons Symposium

The Annual BioZone Research Symposium, organized by the BioZone Leadership Council, was held on November 18, 2016. BioZone students displayed their work in 15-minute, 3-minute, and poster presentations to over 60 attendees. To conclude the day, prizes were awarded as follows:

Best 15-Minute Presentation: Peter Stogios
Best 3-Minute Presentation: Kart Kanger
Best Poster Presentation: Fakhria Razeq

On August 8, 2016, over 80 attendees from BioZone gathered at the BioZone Horizons Symposium. This one-day symposium connected current BioZone members with leaders in biotechnology research (in academia and industry), research product commercialization, and those well-established in other careers, most of whom are BioZone alumni. This event was an opportunity to learn of different career experiences and discuss the wide range of career opportunities open to current BioZone members via panel discussions and entrepreneurship talks. The symposium also provided a platform for strengthening the network within the BioZone community and featured a poster competition, followed by a pub night social at O'Grady's.

iGEM Toronto

BioZone continues to support undergraduate researchers through providing research space to the University of Toronto's 2016 iGEM team "iGEM Toronto." iGEM's (International Genetically Engineered Machine Foundation) mission is to promote the advancement of synthetic biology, and the development of an open community and collaboration.

The iGEM Toronto team consists of undergraduate students who are working on a summer project that will be entered into the iGEM Jamboree in October at Massachusetts Institute of Technology of Technology. The team developed a synthetic biological sensor for detection of gold using a cell-free paper based construct using the gold-sensitive transcriptional activator, GolS, and a reporter gene.

2016 U of T Biomod Team

The UofT Biomod team was comprised of undergraduate students preparing a bio-nanotechnology project to compete at BIOMOD, an annual bio-molecular design competition hosted by the Wyss Institute for biologically-inspired engineering at Harvard University. The purpose of this competition is to challenge undergraduate students from around the world to use their creativity to engineer biomolecules on the nanometer scale and present their findings at the annual Biomod Jamboree. Past projects have focused on bimolecular robotics, logic and computing, and structural bionanotechnology. The UofT Biomod Team has worked on a bionanotechnology design for a multi-drug carrier built with DNA origami and will be testing its functions using gold nanoparticles as a payload. The design was developed and characterized through wet lab experiments and imaging analysis conducted in BioZone labs and Christopher Yip's lab from the IBBME department.

BIOMOD

BioZone Undergraduate Researchers Wins at UnERD 2016

On August 9, Onasvi Kharsikar (1T8), a summer research-student in Professor Grant Allen's Bioprocess Engineering Lab supervised by Dr. Yaldah Azimi (MASc 0T8, PhD 1T3), won two medals at the 2016 Undergraduate Engineering Research Day (UnERD). This annual one-day research conference held at the University of Toronto is hosted by students from the Faculty of Applied Science & Engineering and gives summer research-students the opportunity to share their research achievements in an academic showcase.

Outreach & Media

Science Rendezvous

On May 7, 2016, the 10th Annual Science Rendezvous was held all over the country. Science Rendezvous is a free event celebrating science and engineering with children and the general public. This year, BioZone, PAPTAC (Pulp and Paper Technical Association of Canada) and Chemical Engineering volunteers ran a booth where over 350 children made paper from pulp that contained seeds that could later be planted helping families connect BioZone research to a familiar context (and help save the bees!).

IndiBio Demo Day

As a member of the IndiBio Program, Ardra Bio Inc., the renewable chemicals company founded by Dr. Pratish Gawand (CEO) and Professor Mahadevan, participated in the IndiBio Demo day on July 14, 2016.

Indie Bio, an accelerator that funds and builds biotech startups, had chosen to support this start-up company in 2016. Ardra Bio was founded by BioZone alumni Pratish Gawand and Krishna Mahadevan to engineer technologies to produce completely petroleum-free, high purity, and sustainable biochemicals for the cosmetics market, as well as for pharmaceuticals and foods.

The IndiBio Demo day is an opportunity for companies hosted by the IndiBio accelerator to pitch their products to over 400 investors.

INDIE BIO
ACCELERATING BIOLOGY

Genomics in the Park

On October 27, 2016, Elizabeth Edwards participated in "Genomics in the Park," an event organized by Ontario Genomics to promote the role that synthetic biology can play in advancing research for the well-being of Ontarians. The event was held at the Legislative Assembly of Ontario and attended by many Members of the Provincial Parliament. The event was accompanied by the release of Ontario Genomic's Think Synthetic Biology Report.

Ontario Genomics

BioZone Mass Spectrometry Facility Open House

On June 29, 2016, the BioZone Mass Spectrometry Facility held an open house to showcase the available services from the lab. The Mass Spectrometry Facility demonstrated their diversity in analytical techniques from proteomics, metabolomics, to small molecule analysis, as well as method development services and expert advice on these techniques.

The purpose of this event was to promote discussion on the potential uses of mass spectrometry in order to solve complex research questions and to promote the development of novel and relevant mass spectrometry protocols.

Topics discussed at the open house included:

- New developments in mass spectrometry-based proteomics, small molecule analysis and metabolomics;
- How mass spectrometry can support research and product development;
- The types of analysis supported at the BioZone Mass Spectrometry Facility;
- Starting material requirements, sample preparation, and protocol development.

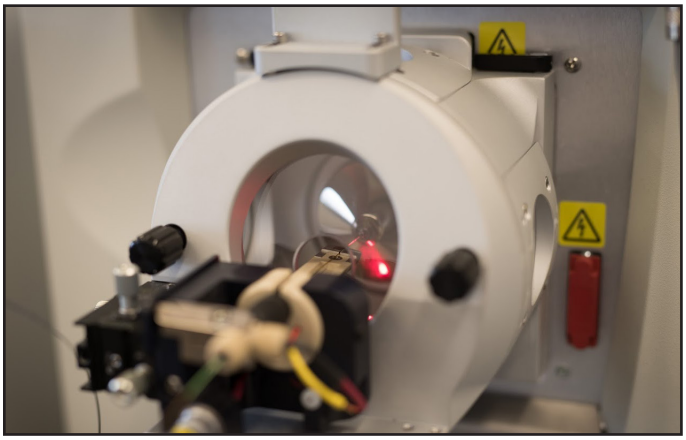
BioZone Research in the Media

Several BioZone researchers and their work were featured in local and national media outlets and publications since 2013:

- *"Paper, not plastic: Leveraging microbial genes to make greener materials",* with Emma Master, U of T Engineering News, December 16, 2016.
- *"Founder Stories: Bringing back industrial biotech with Pratish of Ardra Bio"*, Medium Magazine, July 11, 2016.
- *"Thanks, Invisible Little Water Filters"*, with Elizabeth Edwards, research2reality.com, May 24, 2016.
- *"2016 Killiam Price Winners Interview",* with Elizabeth Edwards, CBC Radio, May 20, 2016.
- *" A tumour you can unroll: engineers create new technology for understanding cancer growth",* with Alison McGuigan and Radhakrishnan Mahadevan, U of T Engineering News, November 23, 2015.
- *"Plant biosensor could help African farmers fight parasitic 'witchweed' ",* with Alexei Savchenko, Phys.org, October 8, 2015
- *"Interview with Brad Saville",* Business News Network, August 28, 2013.
- *"Alberta looks to become Canada's Biofuel Leader",* with Bradley Saville, Edmonton Journal, August 19, 2013.
- *"Childbirth: U of T professor's grant could curb maternal death — with tea",* with Levente Diosady, Toronto Start, July 31, 2013
- *"Keeping all the balls in the air"*, with Elizabeth Edwards, ACCN Canadian Chemical News, March 2013.

Visitors

In addition to hosting a number of guest speakers throughout the year, visiting scholar Professor Savia Gavazza from the Univerisidade Federal de Pernambuco (Brazil) continued her collaboration with BioZone for a year-long sabbatical in Toronto.



Social & Team Events

BioZone Fall Retreat

The Biozone Fall Trip took place on October 21, 2016 beginning at Mono Cliffs Provincial Park and ending at Spirit Tree Estate Cidery. Prepared to brave the rainy weather, the group went on an incredibly scenic 2 hour hike covering approximately 7km of trails. The main highlight was the viewing platform at the top of the 30m cliffs where the vibrantly coloured forest appeared to be endless. After working up an appetite, the next stop was Spirit Tree Estate Cidery in Caledon where we enjoyed hot cider, freshly baked bread, charcuterie, and good company. The place was beautiful and we highly recommend it as a quaint escape not too far from the city.

Holiday Party

The annual potluck holiday party was once again a smashing success. Students organized a fantastic spread of international food, drawing on the culinary skills of our multicultural group.

Tea Time

Every Tuesday at 3pm, students, post-docs, staff and professors gather for tea and cookies at this popular event that fosters friendship, collaboration and discussion among BioZone's many researchers and labs. Ten to twenty BioZone members regularly attend the weekly event, but this number skyrockets when monthly birthday parties are celebrated with cake!

BioZone Association

The BioZone Association was launched in 2016, led by the BioZone Council. The purpose of the BioZone Association is to represent BioZone students, post-doctoral fellows, research assistants, and technicians and to promote a positive and productive work environment.

BioZone Association's Mandate:

Collaboration

To maintain a collegial and collaborative atmosphere within BioZone by encouraging social activities, events, active communication, and knowledge exchange.

Mission

To help shape the goals and mission of BioZone.

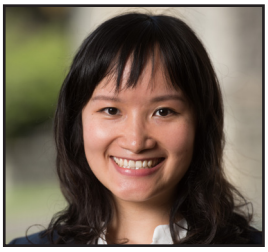
Promote

To promote BioZone as a leading research centre to academia (students, researchers, and collaborators) and industry, through a robust presence on external media such as professional social networks, the BioZone website, and through external events.

Efficiency

To promote efficient operation and maintenance of important shared BioZone equipment and facilities.

The 2016 BioZone Council



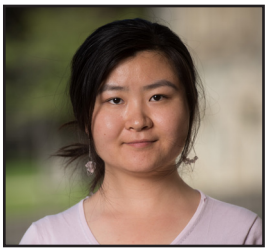
Mabel Wong
President

Elisse Magnuson
Internal Communications Coordinator

Julie-Anne Gandier
External Relations Coordinator

Fei Luo
Academic Development Coordinator

Peter Stogios
Academic Development Coordinator



Ruoyu Yan
Industrial Relations Coordinator

Nigel Guilford
Senior Advisor

Sean Caffrey
Ex-Officio

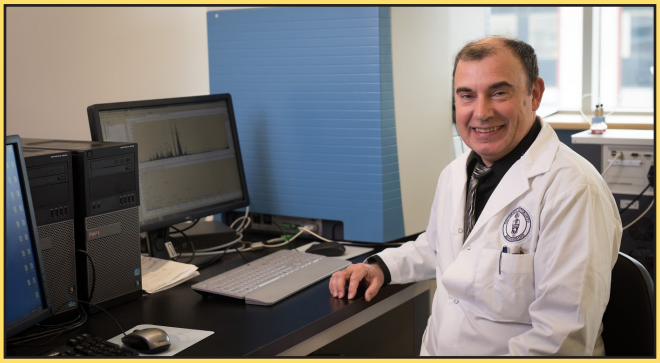
We wish to thank many industrial partners, some of whom are shown below, who have supported our ongoing research.

We would like to thank the public sector agencies who continue supporting BioZone researchers.

BioZone Researchers

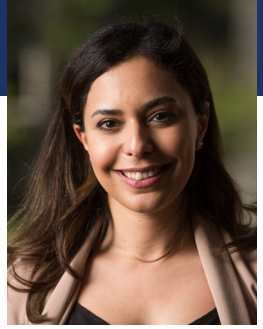
BioZone is home to over ninety students, postdoctoral fellows, research associates and technical staff who continually strive for excellence and innovation in their ongoing research endeavours.

The following pages highlight some of the individual accomplishments and groundbreaking work happening in BioZone.



Research Highlights

S. Kalami, M. Arefmanesh, E. Master, M. Nejad. (2017). Replacing 100% of phenol in phenolic adhesive formulations with lignin. *J. Appl. Polym. Sci.*



Maryam Arefmanesh
Ph.D. Candidate

Supervisor: Emma Master

Chemo enzymatic modification of lignin for resin applications

Lignin is the most abundant natural aromatic polymer and is obtained as a byproduct of biofuel and pulp and paper industries. Lignin has been viewed as a low-value byproduct of these industries and its primary usage is to be burned for local power generation. Increasingly, however, there is interest to develop a broader range of products from lignin that also provide environmentally friendly and cost-effective alternatives to petroleum derived compounds.

Lignin structure and chemistry differs depending on the botanical source and extraction method. Therefore, full characterization of lignin is required before determining which lignin is better for which application.

Lignin has both phenolic and hydroxyl groups in its structure and depending on the ratio of these groups, the properties of lignin can be different. A major limiting factor to broader use of lignin is inaccessibility of hydroxyl functional groups embedded in the lignin structure. Accordingly, lignin modification is necessary to increase the number of hydroxyl groups or to make them more accessible. For example, chemo-enzymatic modification of lignin can generate good candidates for applications in polyurethane and phenolic resins. In addition, by using enzymatic pathways, we can link the modified lignin with bifunctional oligosaccharides to make 100% bio-based resins.

Amir Arellano Saab
Ph.D. Candidate

B.Sc., 2014, Universidad Simon Bolivar
M.Sc., 2016, University of Calgary

Supervisor: Alexei Savchenko
Co-Supervisor: Peter McCourt

Strigolactone receptors in *Striga hermonthica*, crystallographic, biochemical, and genetic studies

Striga spp. (Witchweed) is an obligate parasitic plant that attaches to the host roots to deplete them of nutrients. In Africa, the most destructive *Striga* species, *S. hermonthica*, parasitizes the major food crops sorghum, rice, millet and maize with yield losses ranging from 30 -100%. Presently, the most common method of *Striga* control is hand weeding, which is time-consuming, labour-intensive and disproportionately carried out by women and children.

There are a number of potential solutions to the *Striga* problem, and breeding approaches are presently the most economically sought method of parasitic weed control. There are examples of improved genetic resistance to *Striga* in rice, sorghum, cowpea, and maize, however, complete resistance has not been attained (*Afr J Plant Sci* 8, 492, 2014). This is most likely due to the high genetic variability that is a consequence of the obligate outbreeding mating system of *Striga hermonthica*. This genetic variation not only results in resistance breakdown but almost certainly determines local host range and virulence. Thus, crop resistance across different parasitic plant populations will be difficult to achieve (*Heredity* 108, 96, 2012). This genetic complexity indicates innovative approaches will be required to eradicate *Striga* that exploit weaknesses in its lifecycle and an understanding of how this parasite evolves and adapts. However, such approaches also require a mechanistic molecular understanding of the *Striga* lifecycle.

The overarching goal of this project is a complete understanding of *Striga hermonthica* SL receptors from the fundamental mechanism of perception to their evolution and population biology. Longer term, this information will be exploited to eradicate *Striga* from Sub-Sahara Africa.

Genetic variation most certainly contributes to SL perception and sensitivity and understanding how this mechanistically occurs is paramount to perturbing the system in ways in which the parasite will not be able to adapt. Biochemical and structural analysis of the *Arabidopsis* and the *Striga* receptors gives us some understanding of which amino acids may be important in determining SL receptors perception (*Science*, 349, 864 (2015), *Science*, 350, 203, (2015)). Comparisons of the low sensitive *Arabidopsis* SL receptor (AtHTL) and the highly sensitive *Striga* ShHTL7 receptor binding pockets identifies 8-10 key amino acids that most likely contribute to SL specificity and responsiveness.

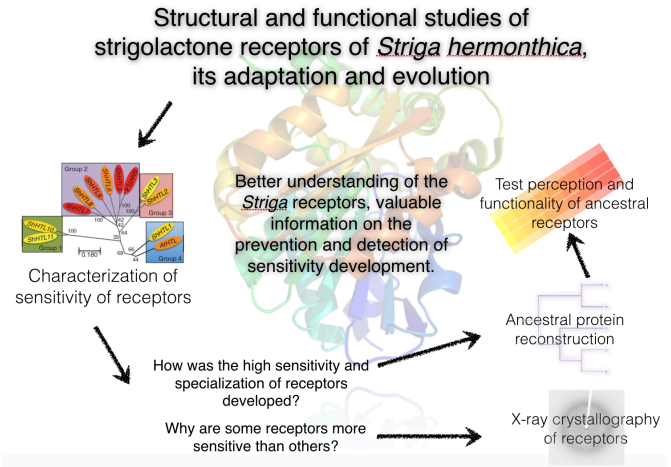


Figure 1: Structural and functional studies of strigolactone receptors of Striga hermonthica.

Research Highlights

A. Arellano. "Crystallographic studies of novel streptavidin mutants in complex with peptide tags", poster presentation at the *Protein Structure, Function and Malfunction Meeting*. Saskatoon, Saskatchewan. May 7-8, 2015.

Full Graduate Scholarship, Mexican National Science of Science and Technology. (2014)

Mitacs Globalink Fellowship Award, MITACS. (2014-2015)

Best Graduate Oral Poster Presentation, Protein Structure, Function and Malfunction Meeting, University of Saskatchewan, Canada. (2015)

Nancy Bawa
Research Assistant

B.Sc. (Hons), 2006, University of Saskatchewan
M.Sc., 2008, University of Saskatchewan

Supervisor: Elizabeth Edwards
Co-Supervisor: Emma Master

Microcosm set-up and analysis for evaluating benzene bioremediation & microbial growth and optimization in bioreactors

Benzene, toluene, ethylbenzene and xylene are highly toxic groundwater contaminants and our goal is to: (a) scale-up cultures that degrade these contaminants anaerobically and, (b) focus on the application of these cultures on site.

Another aspect of my research involves optimizing growth of microbial cultures in bioreactors with a goal to scale-up for enhanced protein production.

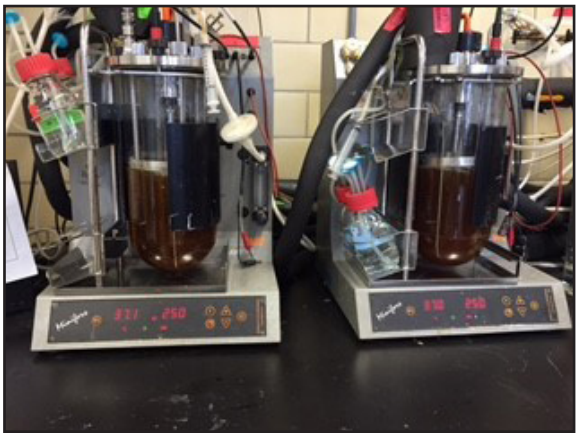


Figure 1: A 5-L bioreactor set-up.

Figure 2: An 80-L bioreactor setup.

Figure 3: Example of a microcosm set-up.

Sofia Bonilla
Postdoctoral Fellow

B.Sc., 2009, Universidad de los Andes
M.Sc.Tech., 2011, University of New South Wales

Supervisor: D. Grant Allen

Proteins for enhancing biosludge dewaterability

Sludge processing and disposal is a challenge due to the high moisture content of biosludge. Chemical conditioners are commonly used to enhance sludge dewaterability, facilitating processing and reducing its volume and thus, disposal costs. However, the chemical conditioners used are petroleum-derived and can be toxic to aquatic systems. Therefore, there is interest in finding more environmentally-friendly conditioners to improve biosludge dewaterability. There have been limited studies on the potential use of enzymes for enhancing the dewaterability of sludge but little is known about the mechanisms for such enhancement. The objective of this project is to better understand how enzymes affect sludge and how these changes affect its dewaterability.

After screening several enzymes, we found that lysozymes improved biosludge dewatering properties. Surprisingly, this effect had nothing to do with the catalytic activity of lysozymes. The positive charge on the surface of lysozymes neutralizes the negative charge of sludge particles leading to larger particles and better dewatering properties. This finding led us to investigate other proteins with a net positive charge and assess their potential for improving sludge dewatering. Also important, these proteins could improve other liquid-solid separations. We found that another protein, protamine, can improve sludge dewaterability. Lysozyme and protamine can also improve other liquid-solid separations such as algal cultures, kaolin and powdered activated carbon suspensions.

Cationic proteins are biodegradable and they can potentially be extracted from waste. Therefore, they offer clear advantages over current chemical or energy-intensive treatments. Furthermore, we now have a better understanding of how to use proteins and enzymes for addressing sludge-related challenges and while doing so, we have developed methodologies to properly evaluate enzymatic treatment of biomass.

Research Highlights

S. Bonilla T, D. G. Allen. (2016). Flocculation with lysozyme: A non-enzymatic application. *Can. J. Chem. Eng.,* **94**, 231-237.

S. Bonilla, H. Tran, D. G. Allen. (2015). Enhancing pulp and paper mill biosludge dewaterability using enzymes. *Water Res.,* **68**, 692-700.

Student Discovery Award, University of Toronto; Dept. of Chemical Engineering. (2017)

W.H. Rapson Memorial Award, University of Toronto; Dept. of Chemical Engineering. (2015)

BioZone Graduate Scholarship, University of Toronto; Dept. of Chemical Engineering. (2012)

Queen Elizabeth II Graduate Scholarship in Science & Technology, Government of Ontario & the University of Toronto. (2012)

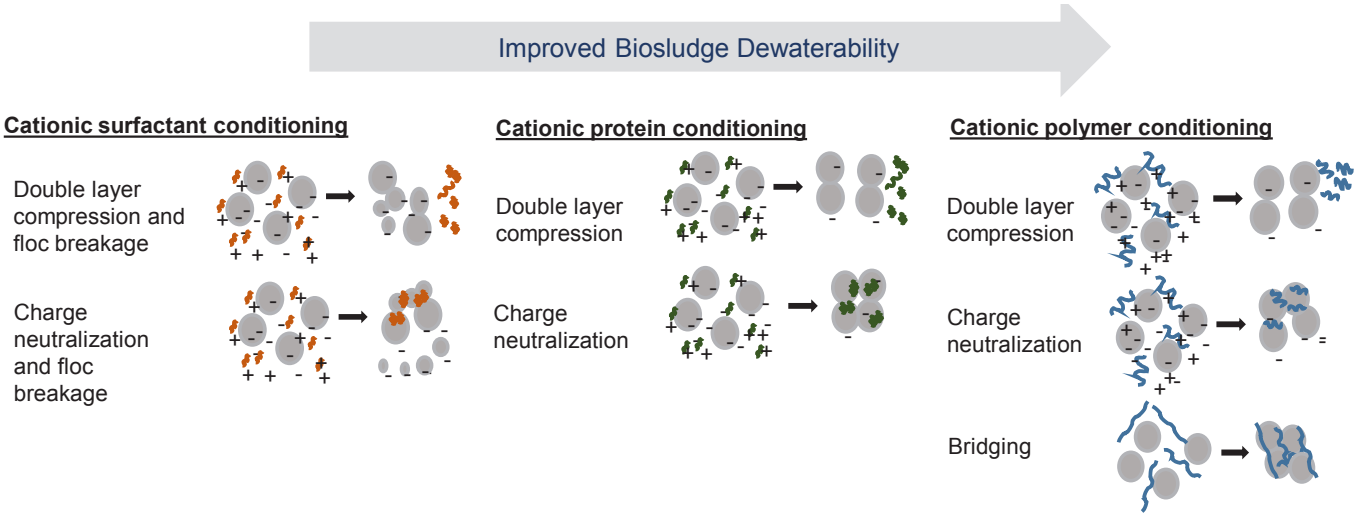


Figure 1: Conditioning mechanisms of surfactants, proteins and synthetic polymers for improving biosludge dewaterability.

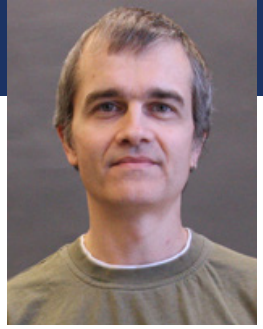
Research Highlights

M. Mimura *et al.* (2016). Arabidopsis *TH2* encodes the orphan enzyme thiamin monophosphate phosphatase. *Plant Cell,* **28**, 2683-2696.

L. Huang *et al.* (2016). A family of metal-dependent phosphatases implicated in metabolite damage-control. *Nat. Chem. Biol.,* **12**, 621-627.

M. Hajighasemi *et al.* (2016). Biochemical and structural insights into enzymatic depolymerization of polylactic acid and other polyesters by microbial carboxylesterases. *Biomacromolecules,* **17**, 2027-2039.

A. Tchigvintsev *et al.* (2015). The environment shapes microbial enzymes: Five cold-active and salt-resistant carboxylesterases from marine metagenomes. *Appl. Microbiol. Biotechnol.,* **99**, 2165-2178.



Greg Brown
Laboratory Technician

H.B.Sc., 1985, University of Western Ontario

Supervisor: Alexander Yakunin

Enzyme discovery, profiling and characterization

Our group has developed a set of general enzyme screens for the functional annotation of purified unknown proteins. Purified proteins are assayed against general chromogenic substrates individually or in pools using 96-well plates and spectrophotometry. We assay for several broad enzymatic activities: phosphatases, carboxylesterases, dehydrogenases, oxidases, proteases and phosphodiesterases.

For proteins with enzymatic activity identified in the general screen, we have a set of secondary screens with which we can determine an enzyme's substrate profile and possibly identify the enzyme's specific or natural substrate. For example, we have a phosphatase screen composed of 95 naturally occurring phosphorylated metabolites (Fig. 1). Activities identified from substrate pools can be deconvoluted to identify the specific substrate utilized by the enzyme.

Once optimal *in vitro* substrates have been identified for a particular enzyme, we do a biochemical work-up; determining optimal pH, temperature, divalent cation requirements and other cofactors. With these parameters we can determine the enzyme's kinetic parameters such as maximal rate of reaction (V_{max}), catalytic constant (k_{cat}) and substrate affinity (K_m) (Fig. 2).

Site-directed mutagenesis (SDM) is used to map the active site of an unknown enzyme. By comparing the amino acid sequence of a putative enzyme to amino acid sequences of known enzymes with similar activities, we choose conserved amino acids and mutate them to Ala or any other amino acid. This is done by changing individual nucleotides (SDM) in the gene encoding the protein. If a particular mutation inactivates the enzyme's activity, that amino acid is part of the enzyme's active site. For the catalytically-active mutant proteins, the analysis of their kinetic parameters can help to determine if these residues contribute to substrate binding.

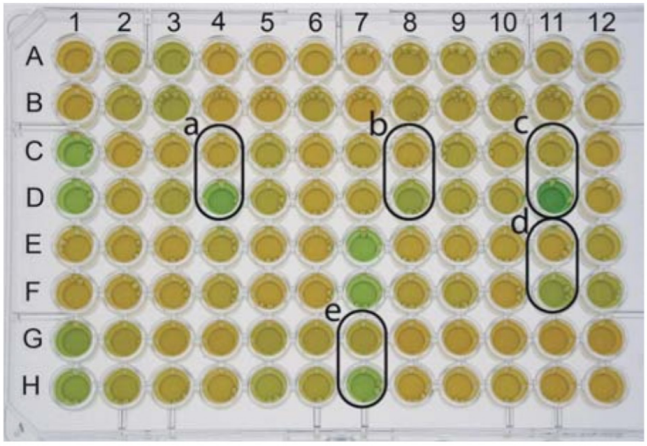


Figure 1: Assay for natural phosphatase activity. Each well contains one of 95 naturally occurring phosphorylated metabolites. A putative phoshphatase, identified in the general screen, is added to each well. If it cleaves the phosphate group from the substrate, the solution will turn green identifying it as a potential cellular substrate of the enzyme.

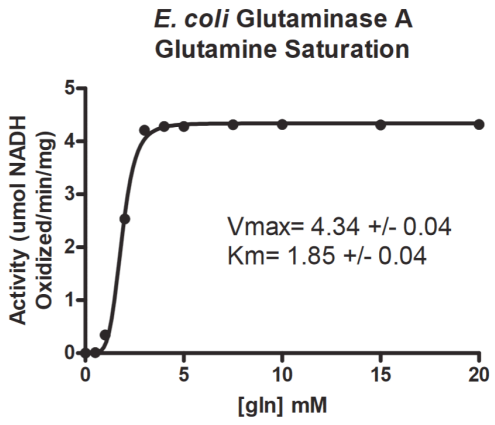


Figure 2: Substrate saturation curve for E. coli Glutaminase A. Glutaminase cleaves an amino group from the amino acid glutamine and is important for cellular nitrogen metabolism.

B.A.Sc., 2013, University of British Columbia

Yee Kei (Kiki) Chan
M.A.Sc. Candidate

Supervisor: Levente Diosady
Co-Supervisor: Yu-Ling Cheng

Research Highlights

Queen Elizabeth II Graduate Scholarship in Science & Technology, Government of Ontario & the University of Toronto. (2017)

Nutrients extraction and fortification using *Moringa oleifera*

Nutrient deficiencies are widespread in populations from both developed and developing nations and have destructive economic impacts as it significantly reduces productivity across the population. Food fortification is used as a method to alleviate nutrient deficiencies. Nutrients are extracted from a nutrient source and added to staple foods to increase the nutrient content of such foods. Research efforts are ongoing in determining suitable fortificants for specific nutrients and food vehicles. Ideally, the fortified product must have the appropriate nutrient dosage, a reliable cost-efficient production process (extraction and fortification), adequate stability, and satisfactory user acceptability based on its colour, taste and smell.

Moringa oleifera is a plant indigenous to tropical and subtropical regions and is densely packed with nutrients such as iron, calcium and magnesium. It appears to be a promising nutrient source as it grows abundantly and is used in local culinary dishes in its native regions. Recent studies have attempted to add *M. oleifera* to bread, cereal gruel, biscuits, yogurts and cheese using powder from ground dried leaves at varying concentrations. At the desired nutrient concentrations, the chief complaint of these fortified products is that they are not as appealing as their unfortified counterpart based on sensory characteristics (i.e., colour, taste, texture and smell). Therefore, there exists an opportunity to extract only the effective nutrient compounds from *M. oleifera* and use them as a fortificant in order to maintain sensory qualities of the fortified foods.

Aqueous and oil extraction techniques for extracting nutrients from plants are well developed and will likely be used in this project. The ideal commercialized production process must be cost effective for developing countries and have relatively simple operations that may be managed by local food producers.

This research will explore the means to reduce common nutrient deficiencies by utilizing a readily available plant, *M. oleifera*, as a source of food fortificants.

Figure 1: Photo of Moringa oleifera leaves

Research Highlights

S. Tabtabaei, B. Hijar, B. K. Chen, L. L. Diosady. (2016). Functional properties of protein isolates produced by aqueous extraction of de-hulled yellow mustard. *J. Am. Oil Chem. Soc.*, **94**, 149-160.

L.L. Diosady, X. Lei, B.K. Chen. US Patent 8,048,463 (2011). "Production of high quality protein isolated from oil seeds."

L.L. Diosady, X. Lei, B.K. Chen. US Patent 6,905,713 (2005). "Production of high quality protein isolates from defated meals of Brassica seeds."

Bih-King Chen
Research Associate

M.A.Sc., 1989, University of Toronto

Supervisor: Levente Diosady

Protein isolation from oilseeds

Development of processes for oil seeds has been one of our group's major research trends. Currently, we are focusing on protein isolation and oil extraction from different varieties of mustard seed. The driving force behind this work is that, despite being a popular food ingredient and condiment, the nutritional value of mustard has not been fully exploited as yet. Furthermore, mustard may also play a substantial role in sustainable energy generation.

In order to produce high-quality protein isolate, it has to go through a series of well-designed unit operations; namely, oil extraction, protein dissolution, chemical treatment, centrifugation, membrane filtration (ultrafiltration and diafiltration), isoelectric precipitation, and drying. Even on the bench scale, all of these individual steps must be optimized and carefully monitored to ensure the desired quality of final product.

Once a bench-scale experimental process has been finalized, scale-up tests are required to obtain engineering parameters that allow:

- determination of the technical and economic feasibility of the process,
- selection of appropriate production-scale equipment,
- optimization of unit operations,
- quality assurance and yield consistency of the final products.

We have done pilot-plant tests in several facilities in Canada and the U.S. The products are excellent binders for meat products. Flavour and texture are indistinguishable between the meat products prepared with the mustard protein isolate and those made with a standard soy protein isolate currently used by the industry. We are now collaborating with a prominent mustard miller for more pilot experiments. The final products' organoleptic properties and their functional properties are to be evaluated.

We found that our process is readily scalable with no spoilage of intermediate products, which demonstrates the process' stability. Moreover, the yield consistency and the quality of the final products proved the technical feasibility of the process.

Figure 1: Membrane filtration units used for our pilot-plant experiments at The Food Protein R&D Center, Texas A&M University, with 300-gal reaction tanks in the background.

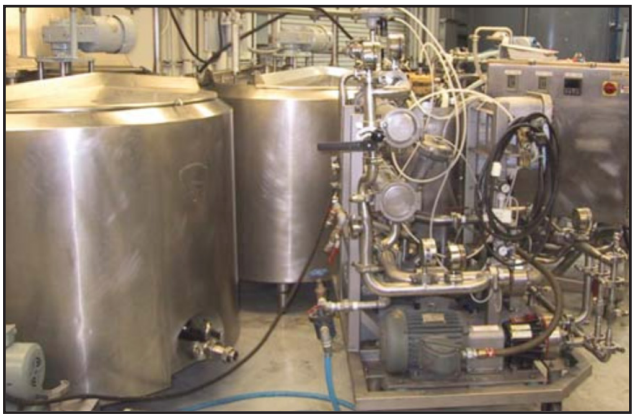


Figure 2: Jacketed stainless steel reaction tanks with 300-gallon capacity, also used as holding tanks

B.Eng., 2015, Zhejiang University of Technology
B.Eng., 2015, University of Western Ontario
M.Eng., 2016, University of Toronto

Xu (Charlie) Chen
Ph.D. Candidate

Supervisor: Elizabeth Edwards

Correlative microscopy in biological samples

Biological samples such as biosludge and microbial culture contain a large amount of different components. It is a mixture of live and dead microorganisms, extracellular polymeric substances (EPS), fibers and inorganic matters. The efficiency of relative treatments such as sludge dewatering and contaminates degradation highly depend on the contributions of the different compositions. The size of those components varies from tens of nanometers to hundreds of micrometers. The visualization study of these components need be carried by optical and electron microscope. The correlative microscopy combines two techniques enabling the use of both optical and electronic analysis techniques on the same sample. For example, optical techniques, such as fluorescence staining, fluorescence *in situ* hybridization (FISH) could be used with SEM and energy dispersive spectroscopy (EDS). However, current conventional sample preparation methods cannot suit to both type of microscopy. Also, the involved dehydration and drying processes for the biological EM sample preparation enables it to present the natural state of sample.

In order to solve these problems, I use a novel correlative sample preparation method — ionic liquid exchanging. The ionic liquid is a conductive "liquid salt" at ambient temperature and also has very low vapor pressure which makes it very stable in the vacuum EM chamber. These properties enable a "wet" sample to be observed in its natural state and also makes it very easy to switch between different microscopy. Currently, an appropriate protocol has been developed and the further study of mixed culture species identification and interaction will be carried on.

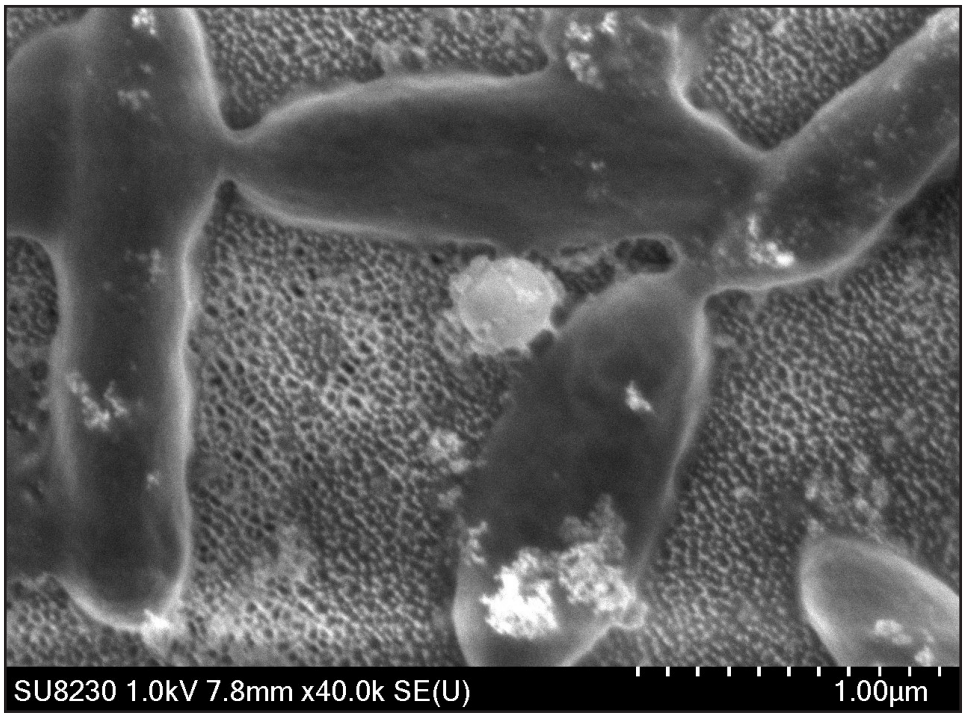


Figure 1: SEM image of benzene degrading culture, ORB1A.

Research Highlights

X. Chen, Y. J. Howe, P. Woo, D. Perovic, E. A. Edwards. "Application of ionic liquid on biological samples in correlative optical microscopy and scanning electron microscopy", invited talk at the *Microscopy & Microanalysis Conference*. Columbus, Ohio. July 26, 2016.

T. Meyer, X. Chen, H. N. Tran, D. G. Allen, E. A. Edwards. (2017). Natural Freezing-Thawing and Its Impact on Dewaterability and Anaerobic Digestibility of Biosludge. *Environ. Eng. Sci.,* **34**, 357-366.

Y. Azimi *et al.* (2013). UV disinfection of wastewater flocs: The effect of secondary treatment conditions. *Water Sci. Technol.,* **67**, 2719-2723.

Research Highlights

NSERC Canada Graduate Scholarships - Master's Program, NSERC. (2016)

Ontario Graduate Scholarship, Government of Ontario. (2015)

Samantha Cheung
M.A.Sc. Candidate

B.A.Sc., 2015, University of Toronto

Supervisor: D. Grant Allen
Co-Supervisor: Steven Short

Attachment surface as a means of manipulating algal biofilm species composition

Microalgae have become a popular area of research for the production of biofuels and bioproducts because of their potential to compete with conventional sources of fuels and materials in a low-cost and environmentally friendly way. In order to be cost competitive with inexpensive products such as fossil fuels, inexpensive feedstocks such as wastewater and flue gas must be utilized in algal systems. Growing microalgae as a biofilm can utilize these inexpensive feedstocks while also reducing the cost of dewatering when compared to planktonic systems. However, non-sterile biofilms contain a variety of different organisms, some of which, may not produce the desired products. In order for algal biofilms to be economically competitive, there needs to be a better understanding of the communities and how to control them. The objective of my research is to determine whether species in algal biofilms can be manipulated by their attachment surface. Molecular biology techniques will be used to quantify biofilm species. This will contribute to the development of genetic techniques and fundamental knowledge in this emerging area of research. Additionally, this will be the first attempt at manipulating biofilm community composition through material. This research has incredible potential for the optimization of algal biofilm product yields where algal species with desired products can be selectively grown over other biofilm organisms. Algal biofilm species selection and control is a necessary step for algal biofuels to become an economically viable source of biofuels and bioproducts.

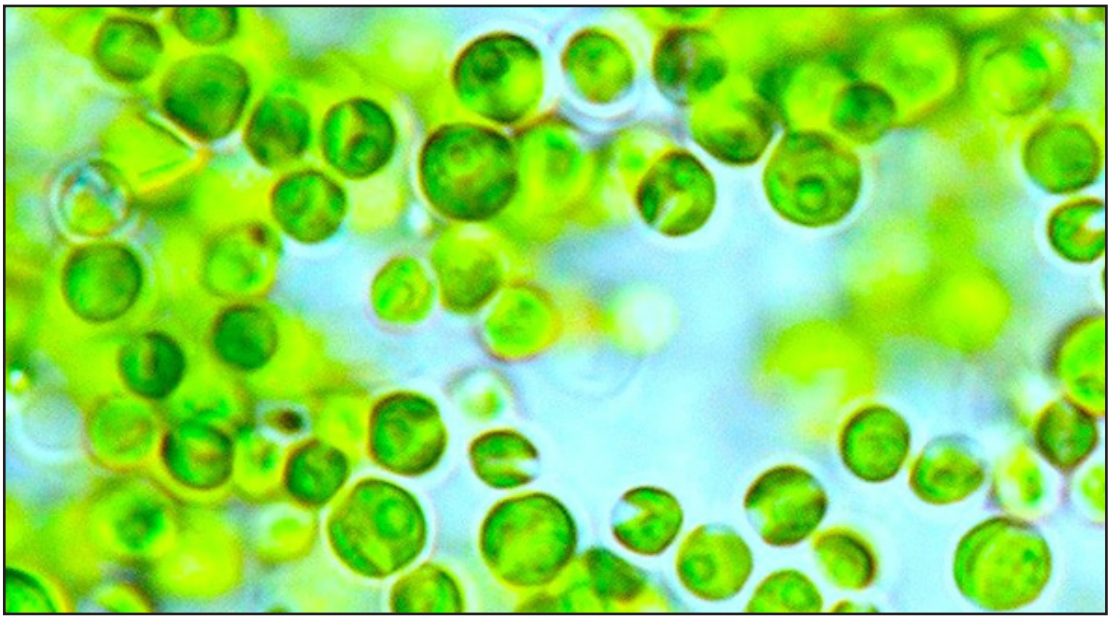
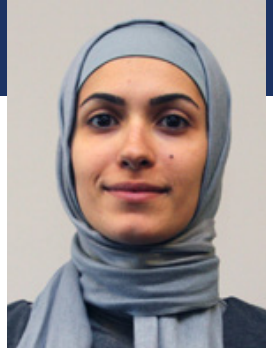


Figure 1: Chlorella vulgaris. This microalgae is commonly used for biofuel production because of its fast growth rates and high lipid content. It is 2-10um in diameter and grows photosynthetically.



Zahra Choolaei
Ph.D. Candidate

B.Sc., 2007, Azad University of North Tehran
M.Sc., 2012, University de Montreal

Supervisor: Alexander Yakunin
Co-Supervisor: Elizabeth Edwards

Enzymatic treatment of pulp and paper mill biosludge

The amount of biosludge generated by wastewater treatment facilities is steadily increasing. In addition, its disposal by landfilling or incineration is very costly and hazardous for the environment. Therefore, it is important to find environmentally friendly and cost effective ways to decrease the ultimate amount of biosludge solids sent for disposal.

Anaerobic digestion can decrease wastewater treatment costs and reduce the amount of produced biosludge to half. It also leads to the production of biogas, which is an alternative energy source to fossil fuels. However, the rate-limiting step of anaerobic digestion is known to be the hydrolysis of organic particulate substances.

Although anaerobic digestion has been widely used for the treatment of wastewater in other industries, it is just recently becoming popular for the treatment of pulp & paper mill effluents. This is due to the presence of some components that slow down the treatment process, such as lignin that is known to be not biodegradable under anaerobic conditions.

To address this issue, pretreatments could be applied to biosludge prior to anaerobic digestion to enhance its digestibility. Our focus in this study is on the enzymatic pretreatment of pulp and paper mill biosludge in advance of anaerobic digestion. Hence, by revealing the problematic constituents of pulp and paper mill biosludge and applying appropriate enzymes on it, we are attempting to improve its anaerobic digestibility.

Research Highlights

Z. Choolaei, A. Yakunin. "Enzymatic pretreatment of pulp and paper mill biosludge for enhancing its anaerobic digestibility", poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Z. Choolaei, S. Bonilla, A. F. Yakunin, D. G. Allen, E. A. Edwards. "Enzymatic pretreatment of pulp and paper mill biosludge for enhancing its anaerobic digestibility", poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.

Helen L. Cross Memorial Graduate Scholarship, University of Toronto; Dept. of Chemical Engineering. (2014, 2015)

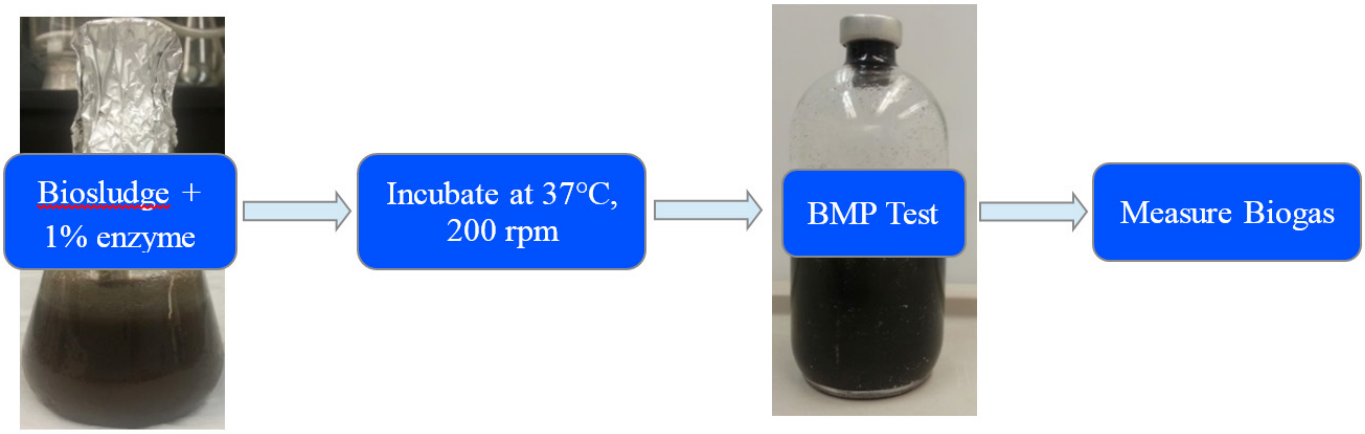


Figure 1: The process of enzymatic pretreatment of pulp and paper mill biosludge, followed by the assessment of its effect on the anaerobic digestibility of the sludge, via BMP assay

Research Highlights

K. Correia, P. Li, L. Yu, R. Mahadevan. “Reconstruction of a yeast pan-genome-scale metabolic model for evolutionary systems biology”, oral presentation at the *17th European Congress on Biotechnology*. Krakow, Poland. July 3-6, 2016.

K. Correia, G. Vemuri, R. Mahadevan. “Modeling pentose metabolism of *Scheffersomyces stipitis*”, oral presentation at the *Pathway Tools Conference*. Palo Alto, California. March 4-6, 2013.

K. Correia, G. Bhutada, G. Vemuri, R. Jeffries, R. Mahadevan. “Elucidating the xylose metabolism of *Scheffersomyces stipitis* using integrated ‘omics analysis’”, poster presentation at the *FIBRE Conference*. Cornwall, ON. May 14-16, 2013.

NSERC CREATE in Manufacturing, Materials and Mimetics (M3), NSERC. (2013, 2016)

Kevin Correia
Ph.D. Candidate

B.A.Sc., 2009, University of Waterloo

Supervisor: Radhakrishnan Mahadevan

Evolution of metabolism in yeasts

Yeasts have been isolated from a wide range of environments across the world. These include wood boring beetles, as pathogens in animals, brine solutions, insect frass, soil, exudate from trees, rotting wood, fermenting juice, and many more diverse environments (Kurtzman et al., 2011). It is clear that yeasts have evolved their metabolism to exploit environmental niches, but specialization can lead to the loss of important traits for biotechnology. For example, yeasts found in sugar-rich environments, such as the skin of ripe fruit, have excellent capacities to ferment hexose sugars to ethanol, but lack the ability to assimilate xylose. In contrast, yeasts often found in the gut of beetles have high abilities to degrade lignocellulose, especially xylose, but have lower tolerance to ethanol and acids. A yeast capable of superior sugar fermentation and superior lignocellulose degradation to ethanol has never been isolated, but would be beneficial for next-generation biofuel and biochemical technologies. Engineering this super yeast is possible if we can understand the detailed mechanisms behind aerobic fermentation (Crabtree-effect) and xylose fermentation, but these mechanisms have been elusive for over 30 years.

If we could study the metabolism of the Proto-Yeast, the mother of all budding yeast in *Saccharomycotina*, along with a Crabtree-positive yeast and xylose fermenter, we could undertake model-based systems biology studies to reverse engineer how Proto-Yeast evolved to become an efficient hexose fermenter or lignocellulose degrader; synthetic biology can then enable us to synthetically breed these traits in a yeast to create superior fermentation performance. The Proto-Yeast is likely extinct, making this study impossible, but its evolved genome lives on in the *Saccharomycotina* family tree. We can’t study the Proto-Yeast’s metabolism, but we can study the metabolism of its living descendants. In recent years, dozens of yeast genomes have been sequenced paving the way for evolutionary systems biology to provide insight into the evolution of metabolism in yeasts.

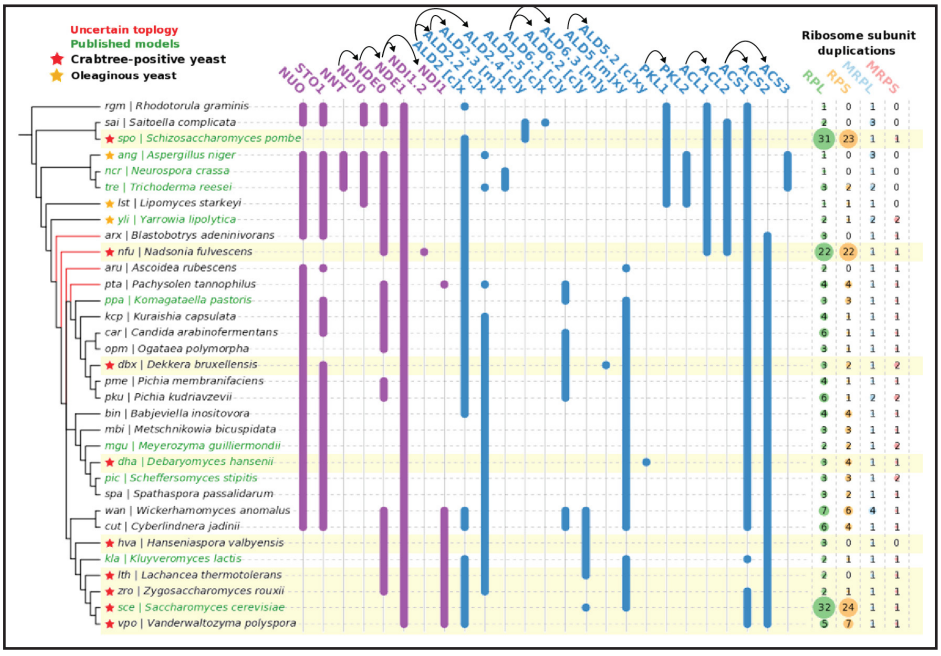


Figure 1: Phylogenetic analysis of enzymes in central metabolism for budding yeasts.

Elisa D'Arcangelo
Ph.D. Candidate

B.Sc., 2013, University College Dublin

Supervisor: Alison McGuigan

Constructing compartmentalized cancer tissues to mimic the tumor edge

Elisa has developed a tool for visualizing how cancer cells mix with surrounding tissue cells in real time. This approach is used to both, understand the dynamics of invasive mixing behaviours and as a platform for performing targeted compound screens.

The utility of this tool is rooted in the understanding that the process of drug discovery would hugely benefit form tools that allow early exclusion of non-viable hits. With this goal in mind, I designed an assay that delivers functional insights within the context of improved relevance to human pathophysiology.

The assay is a custom-built culture device that is used as a functional screen for the emergence of invasive phenotypes in squamous carcinoma cell populations under different culture conditions (specific compounds or presence of cell populations). Compared to conventional *in vitro* invasion assays, such as the spheroid or vertical gel invasion assays, it proves to be advantageous with respect to real-time imaging, compatibility with performing targeted molecular screens, as well as in terms of ease-of-use.

Improved physiological relevance is given by the architecture imposed upon the cancer cell population: embedded in a hydrogel, it is placed adjacent to (as opposed to mixed with) a stromal cell population, such as fibroblasts. This results in a micro-tissue that mimics the dispersive leading edge of a tumor mass, where carcinoma cells interact directly with the neighboring stroma.

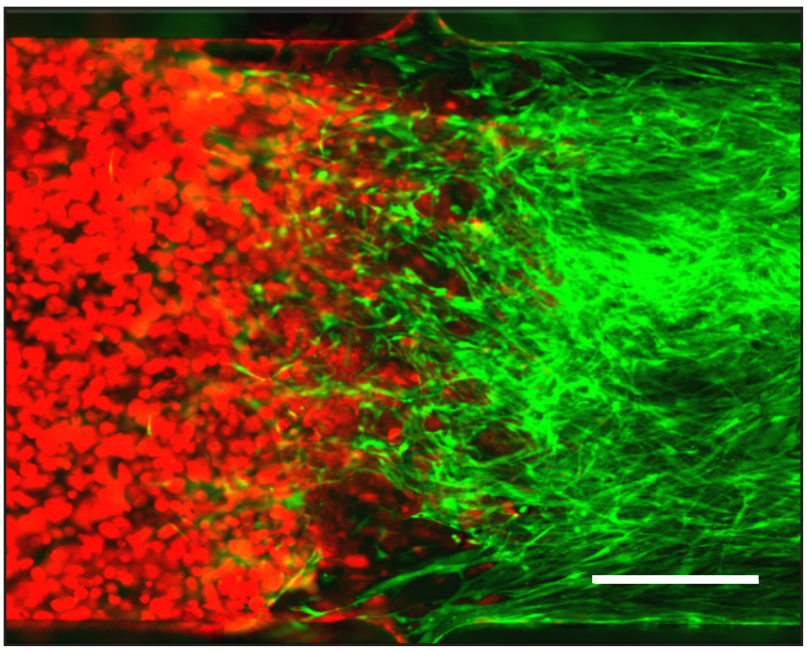


Figure 1: Micrograph showing reciprocal invasion of carcinoma cells (red) and fibroblasts (green), obtained in real time within the culture device (scale bar is 500um).

Research Highlights

E. D'Arcangelo, A. McGuigan. "Compartmentalized hydrogels: A toll for visualizing tumor-stroma interactions", oral presentation at the Biointerfaces Gordon Research Conference. Les Diablerets, Switzerland. 2016.

E. D'Arcangelo, A. P. McGuigan. (2015). Micropatterning strategies to engineer controlled cell and tissue architecture *in vitro*. *BioTechniques,* **58**, 13-23.

S. Javaherian *et al.* (2015). An *in vitro* model of tissue boundary formation for dissecting the contribution of different boundary forming mechanisms. *Integr. Biol.,* **7**, 298-312.

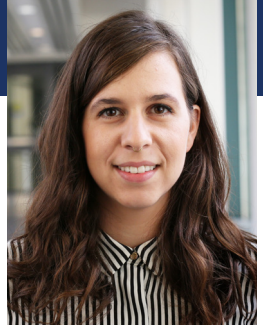
E. D'Arcangelo, M. A.P. "Engineering organotypic tumor microenvironment models", oral presentation at the *International Society of Cancer Metabolism Annual Meeting*. Venice, Italy. September 16-19, 2015.

Ontario Trillium Scholarship, Government of Ontario. (2013-2017)

Research Highlights

NSERC CREATE in Manufacturing, Materials and Mimetics (M3), NSERC. (2016)

NSERC CREATE in Manufacturing, Materials and Mimetics (M3), NSERC. (2017)



Teresa Dean
M.Sc. Candidate

B.Sc. (Hons), 2013, Western University

Supervisor: Alison McGuigan

The effect of cancer associated fibroblasts on tumour cells

In my research project, I use a novel 3D culture platform to investigate the ability of a stromal cell type to promote tumour progression. The assessment of drug effectiveness in cancer often takes place in 2D cell culture models, due to ease of analysis and cost efficiency. However, 2D cultures do not recapitulate the heterogenous tumour microenvironment or 3D architecture of tumours, both of which have an effect on tumour cell behavior. In order to recapitulate these characteristics in a simple *in vitro* model, our lab has developed a novel 3D cell culture platform. Tumor complexity is recreated in this model by incorporating both an extracellular matrix (ECM) component and a cancer stromal cell type (cancer associated fibroblasts). Both the presence of cancer associated fibroblasts (CAFs) as well as an increasing ECM stiffness have been implicated in the progression to an invasive phenotype in tumour cells; my research investigates the interplay of these factors.

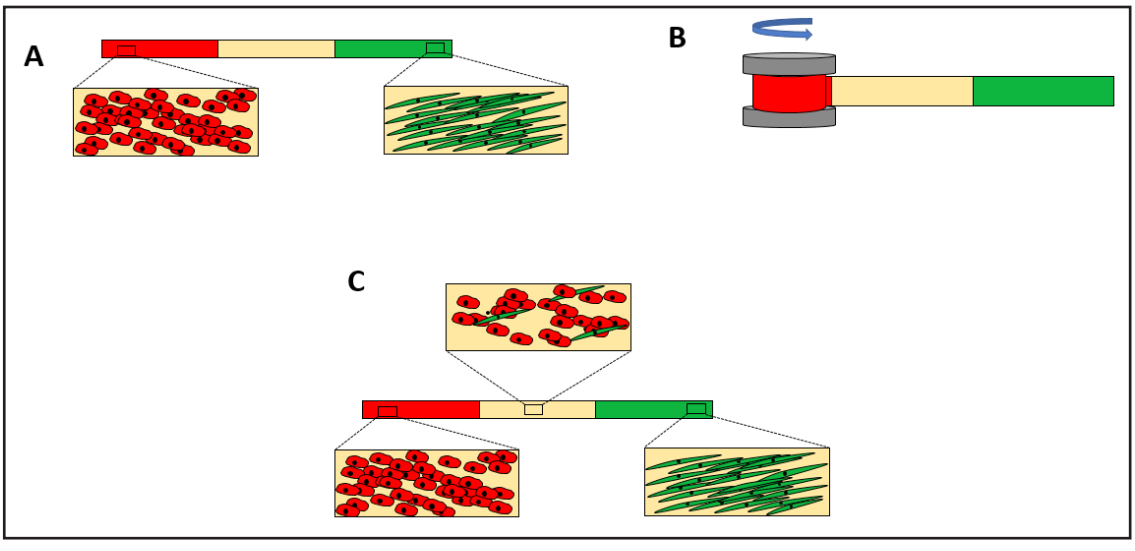


Figure 1: Using TRACER to explore the relationship between tumour cells and a tumour-supporting cell type, cancer associate fibroblasts (CAFs). In cancer, tumour invasion is a prerequisite to metastasis, which often leads to fatality. We are therefore interested in exploring the mechanisms by which tumour cells invade the surrounding tissue. Cancer associated fibroblasts (CAFs), a cell type found in abundance within the tumour stroma, have been demonstrated to play a role in promoting tumour cell invasion. A) Tumour cells (in red) and CAFs (in green), are patterned in the biocomposite. B) The biocomposite is rolled around an aluminum mandrel to assemble TRACER. C) After unrolling, it is observed that some cells have invaded into the inner layer which was initially acellular. Due to TRACER's built-in sectioning capability, we have the unique ability to isolate invasive tumour cell populations and to explore their phenotype.

Rosa Di Leo
Laboratory Technician

B.Sc., 2002, Brock University
M.Sc., 2004, University of Guelph

Supervisor: Alexei Savchenko

Determining the structure of bacterial proteins for insights into pathogenic bacteria

Performs cloning, mutagensis and test expression for assisting in the structure determination of a variety of bacterial enzymes that are involved in mediating resistance to the actions of antimicrobials, with the goal of gaining insights into therapeutic intervention. This is a collaboration with the Center for Structural Genomics of Infectious Diseases (CSGID). In addition, we study proteins of unknown function as a means to discover and their molecular mechanisms and involvement in virulence. Such information will be useful in understanding the basic biological mechanisms of pathogenesis and for the possible discovery of novel antimicrobial drug targets against various pathogen of interest by our group and collaborators.

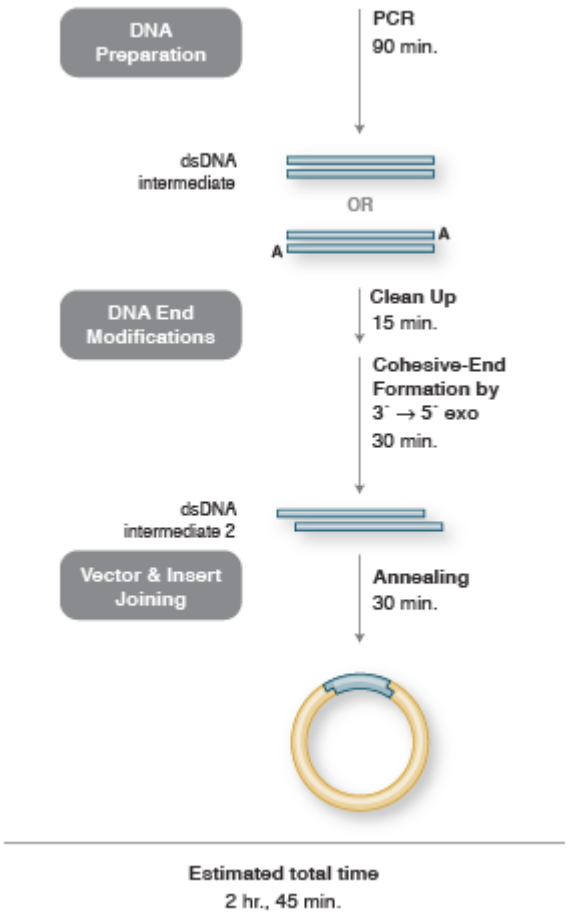


Figure 1: T4 ligation independent cloning procedure.

Research Highlights

W. Wang *et al.* (2016). Biochemical and structural characterization of a five-domain GH115 α-Glucuronidase from the marine bacterium *Saccharophagus degradans* 2-40T. *J. Biol. Chem.,* **291**, 14120-14133.

M. L. Urbanus *et al.* (2016). Diverse mechanisms of metaeffector activity in an intracellular bacterial pathogen, *Legionella pneumophila*. *Mol. Syst. Biol.,* **12**.

E. S. Nakayasu *et al.* (2015). Identification of *Salmonella* typhimurium deubiquitinase SseL substrates by immunoaffinity enrichment and quantitative proteomic analysis. *J. Proteome Res.,* **14**, 4029-4038.

A. P. Kaur *et al.* (2015). Functional and structural diversity in GH62 α-L-arabinofuranosidases from the thermophilic fungus *Scytalidium thermophilum*. *Microb. Biotechnol.,* **8**, 419-433.

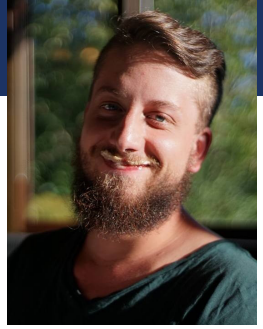
Research Highlights

C. K. Euler, R. Mahadevan. (2016). Protein-level control of metabolism: Design principles and prospects from a representative system. *IFAC-PapersOnLine,* **49**, 165-170.

C. K. Euler, R. Mahadevan. "Protein level control of metabolism: Design principles and prospects from a representative system", oral presentation at the *6th IFAC Conference on Foundations of Systems Biology in Engineering*. Magdeburg, Germany. October 9-12, 2016.

C. K. Euler, K. Mahadevan. "The need for speed (and precision): Protein-level control in a model system", oral presentation at the *Quebec-Ontario Biotechnology Meeting*. Waterloo, ON. May 26-27, 2016.

NSERC CREATE in Manufacturing, Materials and Mimetics (M3), NSERC. (2017)



Christian Euler
Ph.D. Candidate

B.A.Sc., 2014, University of Ottawa
H.B.Sc., 2014, University of Ottawa

Supervisor: Radhakrishnan Mahadevan

Protein-level control of metabolism

I am primarily interested in examining native biological design principles for metabolic regulation at the protein level (i.e., allosteric regulation) to develop optimization tools and techniques for metabolic engineering. Ultimately, my aim is to build fast, continuous control systems for the rational redirection of metabolic flux toward valuable products in microbial cell factories. I do this in two ways. On the fundamental side, I use bioinformatic tools to construct and analyze protein-level regulatory networks to elucidate the design rules for metabolic control. On the applied side, I build model allosteric proteins and characterize their dynamics in metabolic systems relative to analogous transcriptional control systems.

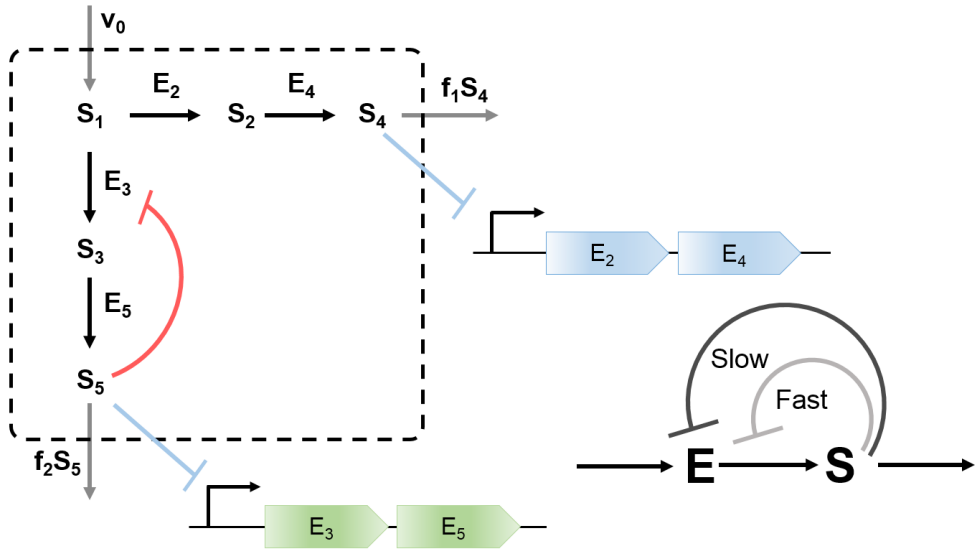
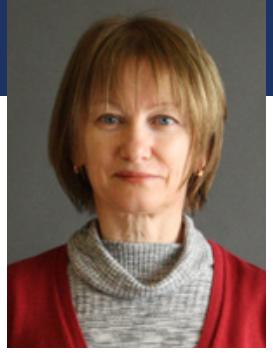


Figure 1: Flux control exists at hierarchical and metabolic levels. Metabolic signals influence transcription and translation of enzymes, as well as enzyme activity directly. This results in fast and slow control loops, the interaction of which I aim to understand.



Elena Evdokimova
Laboratory Technician

M.Sc., 1983, Moscow Veterinary Academy

Supervisor: Alexei Savchenko

Expression, purification and crystallization of proteins for the determination of their 3-D structure by methods of x-ray crystallography

An important use of three-dimensional structural information of proteins is to uncover clues to a protein's function that are not detectable from sequence analysis or to get a more detailed understanding of functional mechanism, if it is not already known.

My main work is production and crystallization of proteins with focus on ligand-bound structures (substrates, co-factors, inhibitors). Getting such structures often requires a lot of work for optimizing both crystallization conditions and quality of protein sample, extensive experience in the area, understanding protein behaviour and knowledge of different techniques and developing more effective routines in protein crystallization.

The targets of my interest include potential industrial enzymes, effector proteins that are involved in the development of infection and proteins, and factors responsible for antibiotic resistance in bacteria.

The extensive use of antibiotics in the treatment of serious bacterial infections has resulted in the emergence of bacterial strains resistant to these antimicrobial drugs. I am involved in studying different classes of antibiotics and their modifying enzymes: aminoglycosides (well-known in antimicrobial treatment such as kanamycin, erythromycin, lincomycin, as well as next-generation plazomicin), glycopeptides (vancomycin), lincozamides (lincomicin, clindamicin), macrolides (azitromicin) and streptogramines- "last-resort" antibiotics for the treatment of infections, caused by Gram-positive pathogens. Particularly, I have been working on crystallization projects for all three known families of aminoglycoside-modifying enzymes: acetyltransferases, nucleotidyltransferases and phosphotransferases. The result of our collaborative work with the Center for Structural Genomics of Infectious Diseases (CSGID) and the Ontario Research Fund (ORF) not only helps to provide insights into evolution, functional and structural plasticity and diversity of enzymes involved in antibiotic resistance, but also gives practical structure-based guidance for finding and optimizing inhibitors to decrease or prevent developing of resistance to already-existing drugs.

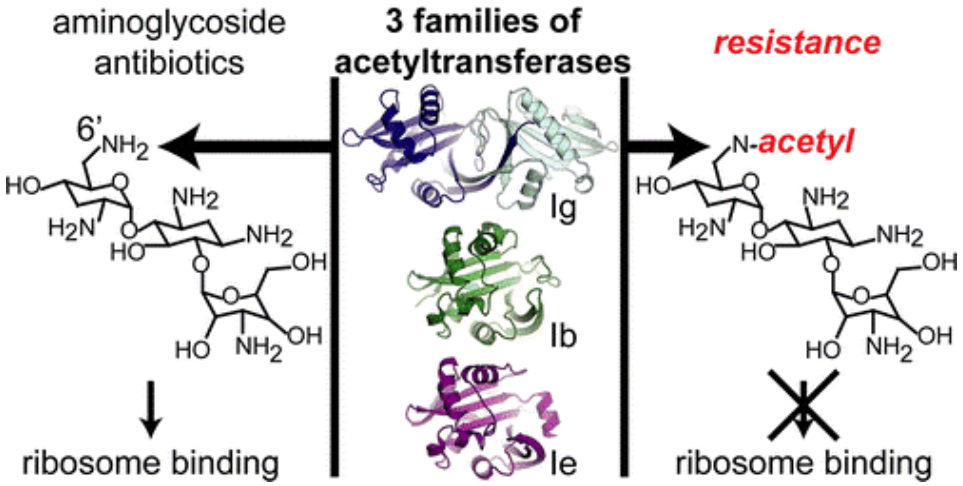


Figure 1: Aminoglycosides are potent antibiotics against both Gram-positive and Gram-negative bacteria, and their bactericidal activity is based on binding to the 30S subunit of the ribosome, leading to protein mistranslation. Modification of aminoglycosides by N-acetyltransferases (AACs) is one of the major mechanisms of resistance to these antibiotics in human bacterial pathogens.

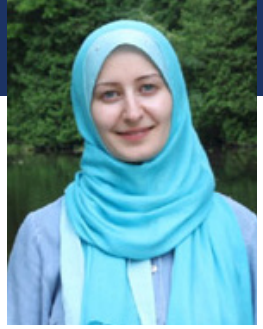
Research Highlights

M. L. Urbanus *et al.* (2016). Diverse mechanisms of metaeffector activity in an intracellular bacterial pathogen, *Legionella pneumophila*. *Mol. Syst. Biol.,* **12**.

N. McGregor *et al.* (2016). Structure-function analysis of a mixed-linkage β-glucanase/xyloglucanase from the key ruminal bacteroidetes *Prevotella bryantii* B14. *J. Biol. Chem.,* **291**, 1175-1197.

P. J. Stogios *et al.* (2015). Structural and functional plasticity of antibiotic resistance nucleotidylyltransferases revealed by molecular characterization of lincosamide nucleotidylyltransferases Lnu(A) and Lnu(D). *J. Mol. Biol.,* **427**, 2229-2243.

M. Morar, E. Evdokimova, C. Chang, A. W. Ensminger, A. Savchenko. (2015). Crystal structure of the *Legionella pneumophila* lem 10 effector reveals a new member of the HD protein superfamily. *Proteins Struct. Funct. Bioinformatics,* **83**, 2319-2325.



Maryam Foumani
Research Associate

B.Sc., 2005, University of Tehran
M.A.Sc., 2007, University of Toronto
Ph.D., 2015, University of Toronto

Supervisor: Emma Master

New applications for engineered carbohydrate oxidases

Genetically engineered carbohydrate oxidases from auxiliary activity family 7, AA7, within the carbohydrate active enzyme database (www.cazy.org) demonstrate advantages over commonly used glucose oxidases when wide substrate profile and H_2O_2 stability is required. For example, such properties make these enzymes especially good candidates for applications in food processing (e.g., bread making), and potentially also biosensors and biofuel cells.

In our recent study, we directly compared the substrate profile and H_2O_2 stability of a commercial glucose oxidase and an AA7 variant that we constructed in the lab, namely CtCBM22A_Y300A. We showed that with the exception of glucose, CtCBM22A_Y300A is over 40 times more active on all tested sugars compared to the glucose oxidase. Moreover, we showed that the presence of the CtCBM22 carbohydrate binding module increased the H_2O_2 stability of CtCBM22A_Y300A during oxidation of glucose.

A preliminary baking trial showed that the addition of CtCBM22A_Y300A enhanced dough elasticity as judged by a skilled baker. In the next step, the rheological properties of the dough and the texture of the bread will be carefully analyzed to quantify the impact of CtCBM22A_Y300A addition.

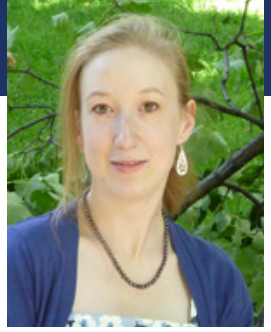
Research Highlights

T. V. Vuong, M. Foumani, B. MacCormick, R. Kwan, E. R. Master. (2016). Direct comparison of gluco-oligosaccharide oxidase variants and glucose oxidase: Substrate range and H2O2 stability. *Sci. Rep.,* **6**.

M. Foumani, T. V. Vuong, B. MacCormick, E. R. Master. (2015). Enhanced polysaccharide binding and activity on linear β-glucans through addition of carbohydrate-binding modules to either terminus of a glucooligosaccharide oxidase. *PLoS One,* **10**.

T. V. Vuong *et al.* (2013). Xylo- and cello-oligosaccharide oxidation by gluco-oligosaccharide oxidase from *Sarocladium strictum* and variants with reduced substrate inhibition. *Biotechnol. Biofuels,* **6**.

M. Foumani, T. V. Vuong, E. R. Master. (2011). Altered substrate specificity of the gluco-oligosaccharide oxidase from *Acremonium strictum*. *Biotechnol. Bioeng.,* **108**, 2261-2269.



Julie-Anne Gandier
Ph.D. Candidate

B.Sc. (Hons), 2010, University of Ottawa
B.A.Sc., 2010, University of Ottawa

Supervisor: Emma Master

The spectrum-wide characterization of hydrophobin proteins: Interface-active proteins with industrial potential

Hydrophobins are secreted non-catalytic fungal proteins that act at interfaces. Their functions range from self-assembling to form a fungal "raincoat" on the fruiting body, to recruiting enzyme activities to surfaces. Such properties have been harnessed in a wide-range of applications from the stabilization of nanoparticles for drug-delivery usages, to coating surfaces for sensing applications and the stabilization of food foams.

My doctoral work has refined the classification of hydrophobins, thus contributing to more predictable structure-function relationships to inform sequence selections for applications purposes. Hydrophobins have been traditionally subdivided into two classes (I and II); however, based on the alignment of hydrophobin sequences predicted from over 200 fungal genomes, we have identified a new subdivision of this protein family: a high-identity group of Class I basidiomycota sequences. To experimentally validate this subdivision, the structure of one of its members, the *Schizophyllum commune* hydrophobin SC16 (hyd1), was solved by solution-NMR. While sharing the core hydrophobin structure, elements believed to be necessary to amyloid-fibre formation, a characteristic of Class I proteins, was absent. We demonstrated, however, that SC16 is capable of forming such structures. This finding allows future studies to address a more general mechanism for assembly.

To further experimentally characterize hydrophobin properties, I tailored techniques such as flow field-flow fractionation to their particularities. While techniques such as small angle x-ray scattering can be applied to determine the average dimensions of assemblies in solution, an upstream separation method is essential to identify multiple oligomeric states and determine their population distribution. Traditionally, size exclusion chromatography is used, however, hydrophobins interact with the separating matrix. Flow field-flow fractionation offers a matrix-free approach to the separation of different oligomeric states that may be present in solution as it uses a perpendicular hydrodynamic force to separate molecules based on geometry and size.

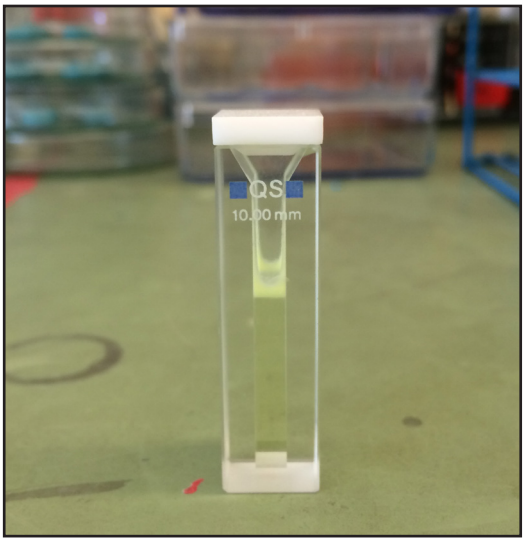


Figure 1: Hydrophobins stabilize foams as can be seen in the superior phase of the solution in the cuvette. The yellow colour in the solution is the dye Thioflavin T used to detect amyloid-fibres. These fibres are an assembled protein state adopted by class I hydrophobins at interfaces.

Research Highlights

J. A. Gandier *et al.* (2017). Characterization of a *Basidiomycota hydrophobin* reveals the structural basis for a high-similarity Class I subdivision. *Sci. Rep.,* **7**.

E. Huynh *et al.* (2015). *In situ* conversion of porphyrin microbubbles to nanoparticles for multimodality imaging. *Nat. Nanotechnol.,* **10**, 325-332.

J.-A. Gandier, E. R. Master. "Bioinformatic and biophysical characterization of a "class III" hydrophobin", oral presentation at the *Gordon Research Conference - Biointerface Science*. Lucca (Barga), Italy. June 15-20, 2014.

The W. Garfield Weston Doctoral Fellowship, University of Toronto; School of Graduate Studies. (2014)

Vanier Canada Graduate Scholarship, Government of Canada. (2011-2014)

Alexander Graham Bell Canada Graduate Scholarship, NSERC. (2011)

Research Highlights

A. Gaona, Y. Lawryshyn, B. Saville. (2015). The effect of fed-batch operation and rotational speed on high-solids enzymatic hydrolysis of hardwood substrates. *Ind. Biotechnol.,* **11**, 277-283.

A. Gaona, Y. Lawryshyn, B. Saville. "Evaluating the mixing performance of high-solids lignocellulosic saccharification through experimental and CFD approaches", poster presentation at the *Mixing XXV Conference*. Quebec City, QC. June 26-July 1, 2016.

A. Gaona, Y. Lawryshyn, B. A. Saville. "Improving high solids enzymatic hydrolysis through reactor design and bioprocess operation", oral presentation at the *World Congress on Industrial Biotechnology*. Montreal, QC. July 23-26, 2015.

Ontario Graduate Scholarship, Government of Ontario. (2012, 2015, 2016)

Adriana Gaona
Ph.D. Candidate

B.A.Sc., 2008, Universidad del Valle
M.Sc., 2012, Ryerson University

Supervisor: Bradley Saville
Co-Supervisor: Yuri Lawryshyn

Exploring the fluid behaviour in high-solids lignocellulosic enzymatic hydrolysis

Ethanol fuel obtained from cellulosic biomass feedstocks has the potential to reduce dependence on fossil fuels. An enzymatic hydrolysis process transforms the biomass into liquid slurry composed of five-carbon and six-carbon sugars, which is then fermented to obtain ethanol fuel. To increase the conversion of fermentable sugars, a high biomass loading in the enzymatic hydrolysis process is required. However, **as the solids concentration is increased, the viscosity of the slurry increases, yielding inadequate mixing in the process.** As a consequence, an industrial scale-up of the process operating at high-solids loading is not yet economically viable.

There has been increased interest to optimize the enzymatic hydrolysis process by studying the rheological properties of various cellulosic biomass feedstocks and conducting experiments in different bioreactor scales to analyze the slurry flow based on empirical correlations. In spite of these efforts, the rheological results are specific to the characteristics of the system studied and are difficult to be implemented to different systems.

This limitation can be overcome by employing computational fluid dynamics, in which the biomass slurry flow can be studied by tuning solids load, and bioreactor dimensions and parameters, such as type and number of impellers, as well as rotational speed. **I plan to develop a computational fluid dynamics model combined with experimental work to understand the biomass fluid behaviour at high-solids loadings.**

Ultimately, we expect that the results of this research will shed light on the relationship between rheological behaviour of the lignocellulosic slurry and the reactor design parameters, in order to promote mixing and optimize the hydrolysis process.

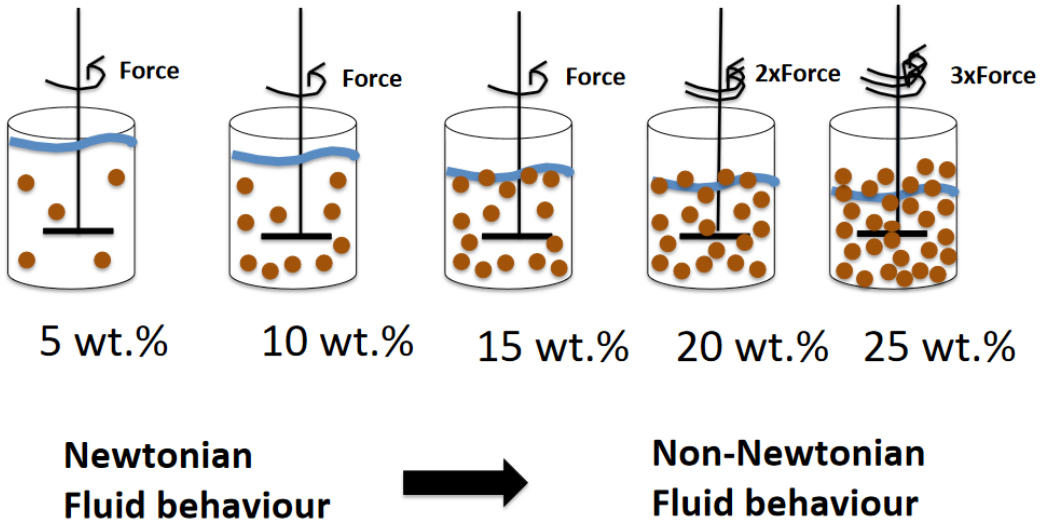


Figure 1: Mixing limitations due to solids content.

Nigel Guilford
Ph.D. Candidate

B.Sc., 1969, University of Leeds
M.Eng., 2009, University of Toronto

Supervisor: Elizabeth Edwards

Anaerobic digestion of organic wastes of variable composition

Problem Statement: More than 13 million tonnes of solid waste is landfilled every year in Canada, where it decomposes anaerobically giving rise to 20 Mt of CO_2eq./year of GHG emissions. In theory most of this organic waste could be anaerobically digested under controlled conditions thereby obviating the emissions problem. In practice, using conventional anaerobic digestion technology, this is prohibitively expensive, largely because of the complex and costly pre-processing required. My research seeks to address this problem.

Knowledge Gaps: The anaerobic digestion of food waste, and the organic fraction of municipal solid waste, have been extensively studied. But this represents only 35% of all solid waste; the remaining 65%, from commercial industrial sources, has been largely ignored. The co-digestion of food waste with other waste products like municipal sludges and crop residues has also been studied but the co-digestion of food waste with hard-to-digest paper products (cardboard, boxboard, newsprint, office paper) has barely been touched.

Addressing the Questions: A novel system for anaerobically digesting the organic component of commercial/industrial solid waste, one which involves very little pre-treatment, has been designed and constructed on a lab scale; it has been operating continuously for 500 days to demonstrate the versatility and robustness of the technology under a range of operating conditions. Biogas production and system temperatures are measured continuously; feedstock and digestate are analyzed on a regular basis; operating conditions within the digester are analyzed four times a week. A powerful synergistic effect of food waste on the digestion of lingo-cellulosic wastes has been found, possibly resulting from enzymatic activity induced by the presence of food waste.

Commercial Prospects: Because of the simplicity of the design, the results obtained in the laboratory, and an initial financial analysis, the technology has good commercial prospects. A demonstration scale version is planned as part of a large commercial waste processing facility planned for Ottawa. The single most important variable is the solids retention time in the reactor.

Role of my Research: The most important contributions of my research are proof of concept, demonstration of robustness and stability under conditions of changing feed stock and identification of key variables to maintain stable operations.

Figure 1: Two-stage anaerobic digester: sequentially-fed leach beds and an up-flow anaerobic sludge blanket reactor.

Research Highlights

H. Lee, N. G. H. Guilford, E. A. Edwards. “Characterization of the microbial community of an anaerobic digester treating solid waste”, poster presentation at the *International Society for Microbial Ecology*. Montreal, QC. August 25, 2016.

N. Guilford. “A Life’s Work... So far: 47 Years in 10 Minutes”, oral presentation at the *BioZone Horizons Symposium*. Toronto, ON. August 2016.

N. Guilford, E. Edwards. “Comparative life cycle inventory analysis of alternative methods for organic solid waste management “, oral presentation at the *A&WMA/ONEIA Conference*. October 7, 2015.

N. Guilford, E. Edwards. “Anaerobic digestion of solid wastes - A new approach”, oral presentation at the *4th Annual BEEM Conference*. February 2014.

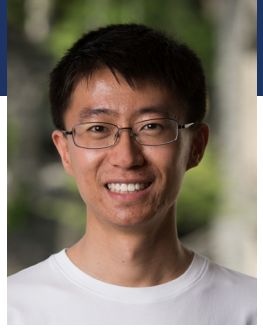
Research Highlights

S. Guo, J. C. Joo, S. Partow, R. Mahadevan. "Biotechnological production of adipic acid", poster presentation at the *Ontario-Quebec Biotechnology Meeting*. Toronto, ON. May 15-16, 2014.

K. Nemr, S. Guo, R. Mahadevan. "A novel computational model-based metabolic engineering strategy for biofuel and biochemical production in *Escherichia coli*", poster presentation at the *Advanced Biofuels Symposium*. Ottawa, ON. May 27-29, 2014.

Connaught International Scholarship, University of Toronto; School of Graduate Studies. (2013-2015)

Departmental Fellowship, University of Toronto; Dept. of Chemical Engineering. (2013-2016)



Shen Guo
Laboratory Technician

B.S., 2013, Brandeis University
M.A.Sc., 2016, University of Toronto

Supervisor: Elizabeth Edwards

Anaerobic benzene degradation in groundwater

Benzene is a component of crude oil which is widely used in the chemical industry. Its accidental spills and releases have caused serious ground water contamination. Environmental microbiology approaches have shown to be an effective way of solving ground water contamination. However, benzene degradation, particularly in the absence of oxygen, is more difficult due to its stable chemical structure, and its mechanism within the microbial community is largely unknown. Recently, industries have shown a keen interest in the anaerobic benzene-degrading cultures in Edwards Lab. The cultures illustrated their potential both for real site application and as rare sources of study for the mechanisms of anaerobic benzene degradation. Our current work has been focused on testing the feasibility of these cultures for industrial uses and the study of the benzene degradation pathways within the cultures.

To test the applicability of the cultures in contaminated sites, a culture scale-up process is required. This work involves maintenance of the cultures in bioreactors and anaerobic glovebox and culture transfer through inoculation. Meanwhile, biotreatability studies are conducted to establish the potential of our cultures. We are also developing qPCR methods to track the essential degraders in the cultures to monitor *in situ* benzene degradation potential. Biomarkers and Next Generation Sequencing-based metagenome screens are used to characterize microbial communities in a wide variety of benzene-contaminated environments. Proteomics and enzyme characterization are used to identify putative enzymes responsible for crucial anaerobic benzene degradation steps.

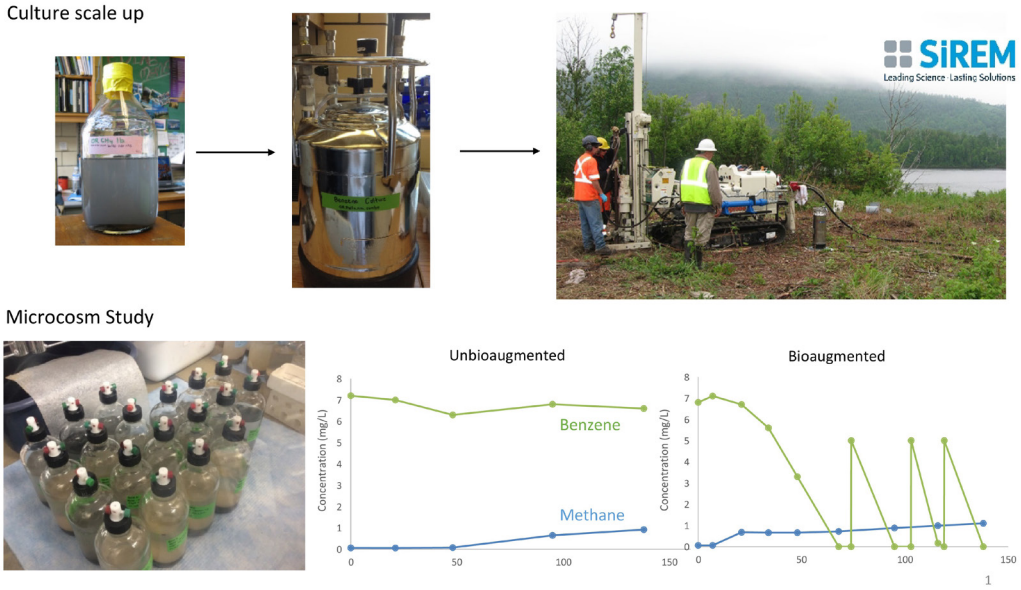


Figure 1: Research overview.

Mahbod Hajighasemi
Ph.D. Candidate

B.Sc., 2004, Azad University
M.Sc., 2007, University of Tehran

Supervisor: Elizabeth Edwards
Co-Supervisor: Alexander Yakunin

Enzymatic depolymerization of synthetic polyesters by microbial carboxylesterases

In response to the increase in social awareness about environmental issues, biodegradable plastics are getting more popular. Several types of biodegradable polyesters with different physical properties have emerged to replace traditional petroleum-based polymers. However, there is no efficient recycling strategy in place for most of these plastics. As a solution, we've developed a patent-pending, enzyme-based technology that not only enables **efficient degradation of polyesters as a recycling approach, but may also serves as a platform to convert post-consumer waste to different value-added chemicals by genetically engineered bacteria.** As opposed to the composting process, enzymatic hydrolysis of bioplastics generates carboxylic acids instead of CO_2 as the final product.

In our work, over 250 uncharacterized α/β-hydrolases from sequenced microbial genomes and metagenomic libraries were recombinantly expressed in *E. coli* and purified using metal (Ni) chelate chromatography. Purified proteins were screened for hydrolytic activity against polylactic acid (PLA), polycaprolactone (PCL), and a model polyethylene terephthalate substrate (3PET). **Multiple rounds of screening yielded 40 active polyester-degrading enzymes, 14 of which were biochemically characterized.** In parallel, the crystal structures of three polyester hydrolases were determined and the active site residues critical for polyester hydrolysis were identified using structure-based, site-directed mutagenesis. The product analyses revealed that polyesters were completely hydrolyzed to water soluble oligomeric species and eventually to monomers. Our results indicate that microbial carboxyl esterases can efficiently hydrolyze various polyesters making them attractive biocatalysts for plastics depolymerization and recycling.

Alternatively, polyester-hydrolyzing enzymes can be used for surface functionalization of bioplastics. Enzymatic hydrolysis of biodegradable plastics creates hydroxyl and carboxyl groups on the surface of polyester material without modifying the properties of the core polymer. The charged groups on the surface contribute to wettability of the polyester and therefore make it more biocompatible for medical and pharmaceutical applications.

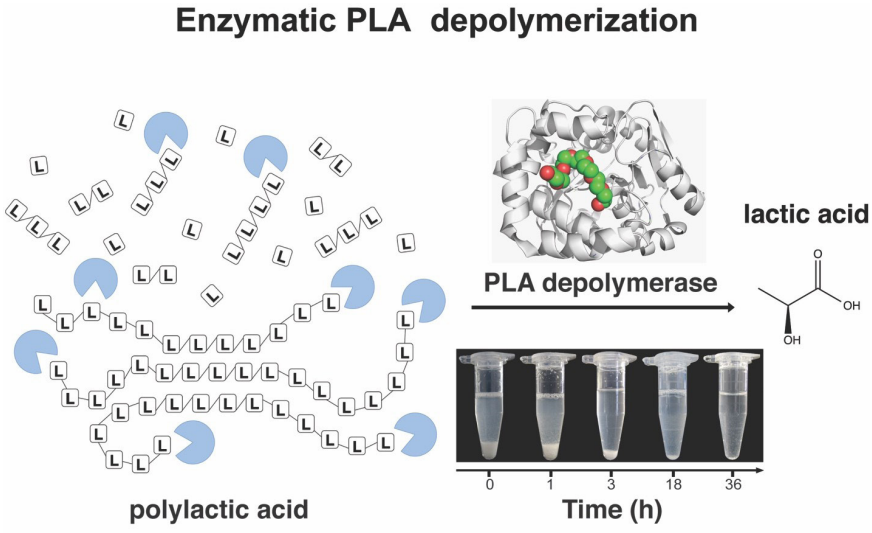


Figure 1: Enzymatic degradation of polylactide, a thermoplastic polyester from renewable resources. The polyesterase enzymes catalyze the hydrolysis of ester bonds releasing water-soluble lactate oligomers and lactic acid monomers as the final product.

Research Highlights

M. Hajighasemi, A.F. Yakunin and E.A. Edwards (2016) Processes, enzymes and microorganisms for depolymerizing polyester plastics. U.S. Patent Application No. 15,223,866, Canadian Patent Application No. 2,937,569.

M. Hajighasemi *et al.* (2016). Biochemical and structural insights into enzymatic depolymerization of polylactic acid and other polyesters by microbial carboxylesterases. *Biomacromolecules,* **17**, 2027-2039.

Doctoral Completion Award, University of Toronto; School of Graduate Studies. (2016)

Frank Howard Guest Bursary, University of Toronto; Dept. of Chemical Engineering. (2015)

Applied Science Graduate Faculty Fellowship, University of Toronto. (2015)

Hedieh Hashtroudi
M.Eng. Candidate

Supervisor: D. Grant Allen
Co-Supervisor: Yaldah Azimi

Investigating the role of select fruit waste enzymes (kiwi and pineapple) on biogas production in anaerobic digestion of municipal sludge

Anaerobic Digester (AD) is the efficient way to reduce the municipal sludge and produce biogas (approximately 70% methane and 30% CO_2) that can be used to generate energy. However, due to rate -limiting hydrolysis processes, sludge pre-treatment is commonly applied to enhance the efficiency of the overall process. Conditioning sludge with enzymes, is one of such pre-treatments that assist COD solubilisation and improves the overall performance of the AD process.

Hence this research, consisting of a simulated anaerobic digester process, is carried out to evaluate the performance of AD by adding fruit enzyme (kiwi, pineapple, papaya) from waste, and also assesses the yield of biogas production.

Spencer Imbrogno
M.A.Sc. Candidate

B.Eng., 2016, McMaster University

Supervisor: Emma Master

Chemo-enzymatic synthesis of oligosaccharide building blocks

Fungi produce enzymes that modify plant fibre (lignocellulose). Many fungal carbohydrate-active enzymes (or CAZymes) have been discovered including Auxiliary Activity (AA) family enzymes galactose oxidase (GalOx) and gluco-oligosaccharide oxidase (GOOX) which oxidize certain oligo- and polysaccharides to introduce aldehydes and carboxylic acids at specific positions. These oxidized sites can then facilitate subsequent targeted chemical modifications to prepare biobased value-added chemicals and materials. Moreover, enzymatic oxidations provide precise modification under mild, cell-free, aqueous conditions, with low quantities of enzyme that retain valued carbohydrate structures.

Lignocellulosic biomass can be used as a sustainable and economical source of chemicals and materials. Hemicelluloses represent ~30% of wood fibre and when used, are typically deconstructed to fermentable sugars for production of fuels and precursor chemicals that forego the value of native biomass structures.

This project describes a chemo-enzymatic approach to upgrade native hemicellulose structures for use as crosslinkers relevant to bio-based resins.

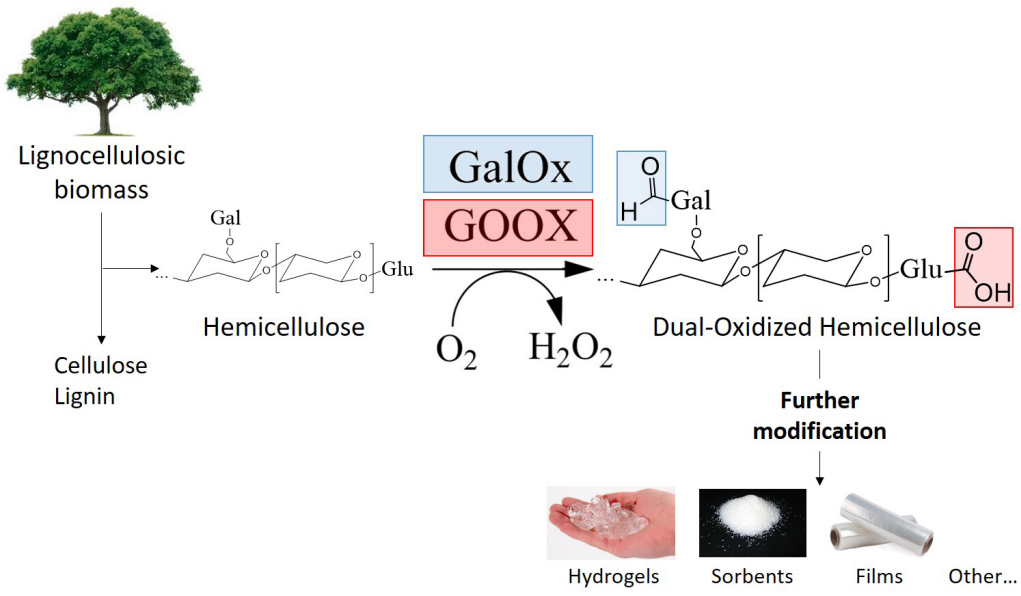


Figure 1: General strategy for dual-enzyme oxidation of hemicellulose for the preparation of value-added products.

Research Highlights

S. Imbrogno, E. R. Master. "Moving toward a single step di-functionalization of plant-derived oligosaccharides using oxidoreductases for value-added bioproducts", poster presentation at the *Biozone Research Symposium*. Toronto, ON. November 18, 2016.

Queen Elizabeth II Graduate Scholarship in Science & Technology, Government of Ontario & the University of Toronto. (2017)

Research Highlights

M. Khaksar Toroghi, W. R. Cluett, K. Mahadevan. (2016). Multi-scale metabolic modelling approach for predicting blood alcohol concentration. *IEEE Life Sciences Letters,* **2**, 59-62.

M. Khaksar Toroghi, W. R. Cluett, K. Mahadevan, paper presented at the 11th IFAC Symposium on Dynamics and Control of Process Systems, Norway, 2016.

M. Khaksar Toroghi, W. R. Cluett, R. Mahadevan. "Multi-scale modelling of whole-human body", oral presentation at the *64th Canadian Chemical Engineering Conference*. Niagara Falls, ON. October 19-22, 2014.

Ontario Graduate Scholarship, Government of Ontario & the University of Toronto. (2016)

Queen Elizabeth II Graduate Scholarship in Science & Technology, Government of Ontario & the University of Toronto. (2015)

Masood Khaksar Toroghi
Ph.D. Candidate

B.Sc., 2006, Ferdowsi University of Mashhad
M.Sc., 2012, École Polytechnique de Montreal

Supervisor: Radhakrishnan Mahadevan

Multi-scale modelling of the whole human body

The multi-scale modelling approach is a powerful mathematical technique for simulating and analyzing complex biological systems such as the human body. This tool can help study the interactions of the various networks in a living organism, from the cellular level up to the population scale, in one framework. In this project, a generic mathematical model is developed that describes human metabolism with 237 serum metabolites integrated with a chosen set of human metabolic networks. A new computational approach is proposed for solving resulting dynamic problem using parsimonious flux balance analysis (pFBA). The aim is to use the developed model in computational medicine, systems pharmacology, and human metabolism study.

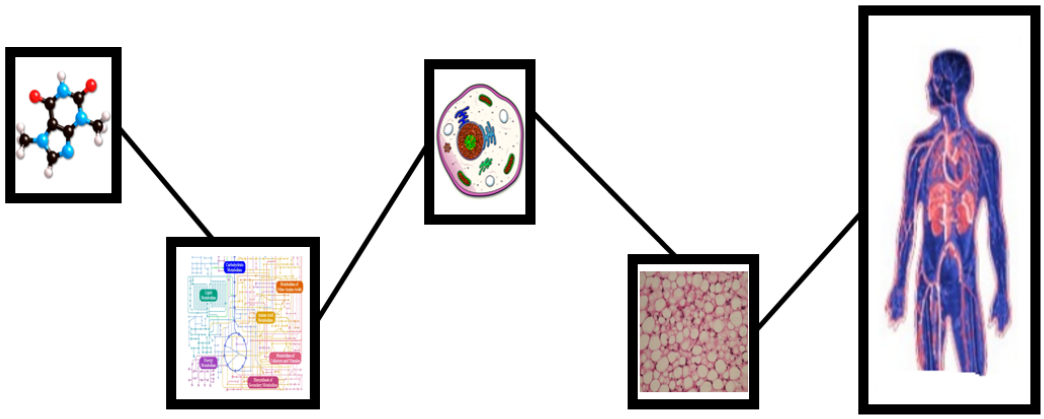


Figure 1: Human body like the other biological systems is considered as a multi scale system. The chemical processes occurring in the body have different time scales from seconds to days.

B.Sc., 2006, University of Tehran
M.Sc., 2009, Sharif University of Technology
Ph.D., 2015, McMaster University

Yaser Khojasteh-Salkuyeh
Postdoctoral Fellow

Supervisor: Bradley Saville
Co-Supervisor: Heather MacLean

Techno-economic and environmental evaluation of hydrogen production technologies from biomass and natural gas

Hydrogen is one of the key chemicals which is used in a variety of processes and technologies. Traditional hydrogen production plants are mostly based on natural gas and other fossil fuels reforming, without any carbon capture and sequestration (CCS) system.

Since H_2 plants are relatively CO_2 intensive, reducing the carbon emissions of the hydrogen production units can significantly reduce the greenhouse gas emissions of the refineries and other energy sectors that consume hydrogen. Besides environmental impacts of hydrogen plants, these units are energy intensive too. Therefore, incorporation of new reforming technologies, utilizing renewable resources (e.g., biomass), and development of novel carbon capture technologies can improve their techno-economic performance and reduce their life-cycle environmental impacts and fossil fuel depletion.

The main objective of this research is to evaluate the techno-economic performance and life cycle energy and environmental impacts of different hydrogen production pathways from biomass and natural gas. Various biomass gasification and natural gas reforming technologies are modelled in this work. The economic performance and environmental impacts of each technology are computed and compared with traditional natural gas-based hydrogen plants at various market conditions. Furthermore, the impact of CO_2 capture and sequestration on the performance of each technology is evaluated. This evaluation is required to understand the potential of these technologies to reduce the environmental footprint of sectors that are connected to hydrogen fuel and determine the best option to enter the hydrogen market. The cradle to gate life cycle assessment are performed for all options, and results are compared with the traditional natural gas-based systems.

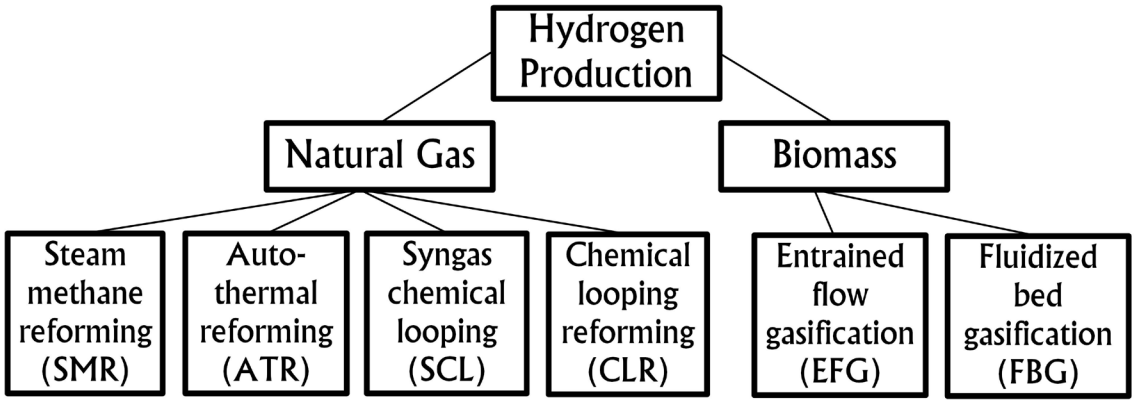


Figure 1: Hydrogen production technologies from natural gas and biomass.

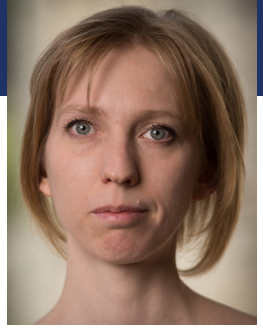
Research Highlights

A. Popovic *et al.* (2017). Activity screening of environmental metagenomic libraries reveals novel carboxylesterase families. *Sci. Rep.,* **7**.

L. Huang *et al.* (2016). A family of metal-dependent phosphatases implicated in metabolite damage-control. *Nat. Chem. Biol.,* **12**, 621-627.

A. Tchigvintsev *et al.* (2015). The environment shapes microbial enzymes: Five cold-active and salt-resistant carboxylesterases from marine metagenomes. *Appl. Microbiol. Biotechnol.,* **99**, 2165-2178.

E. Kuznetsova *et al.* (2015). Functional diversity of haloacid dehalogenase superfamily phosphatases from *Saccharomyces cerevisiae*: Biochemical, structural, and evolutionary insights. *J. Biol. Chem.,* **290**, 18678-18698.



Anna Khusnutdinova
Research Associate

Ph.D., 2012, Institute of Basic Biological Problems, Russian Academy of Sciences

Supervisor: Alexander Yakunin

Enzyme characterization for industrial application

Recent works in biocatalysis and metabolic engineering have enabled the construction of several highly efficient microbial cell factories for conversion of renewable resources to valuable chemicals, such as aliphatic dicarboxylic acids and diols. They can be applied as monomer building block chemicals that are used to produce plastic materials or as intermediates for chemical industry.

One of the most common synthetic polymers is nylon, which represents a family of polymers produced using adipic acid (AA), hexamethylenediamine (HMD) and 6-aminocaproic acid (AC). Traditionally, the monomers required for synthesis of these polymers have been derived from non-renewable petroleum. Further progress towards production of renewable "biomonomers" is hindered by a limited choice of available enzymes and pathways.

The main goal of my project is to identify novel enzymes for the biosynthesis of adipic acid and other polymer precursors from renewable sources. We have demonstrated a broad substrate spectrum of purified bacterial enoate reductases (ERs, EC 1.3.1.31), which can hydrogenate aromatic and aliphatic 2-enoates like muconic acid and 2-hexenedioic acid to adipic acid with a high conversion rate and yield. We also identified and characterized several carboxylic acid reductases (CARs, EC 1.2.99.6), for bioconversion of adipic acid when coupled with aminotransferases (ATs, EC 2.6.1.48; EC 2.6.1.19), or alcohol dehydrogenases (ADs, EC 1.1.1.-) to HMD, AC, 1,6-hexanediol, and 6-hydroxyhexanoic acid. Finally, we identified several CARs and ADs for the *in vitro* and *in vivo* biotransformation of 4-hydroxybutyrate to 1,4-butanediol. For *in vitro* reactions, we have established several cofactor regenerating systems including ATP, NADPH, NADH and amino-donors.

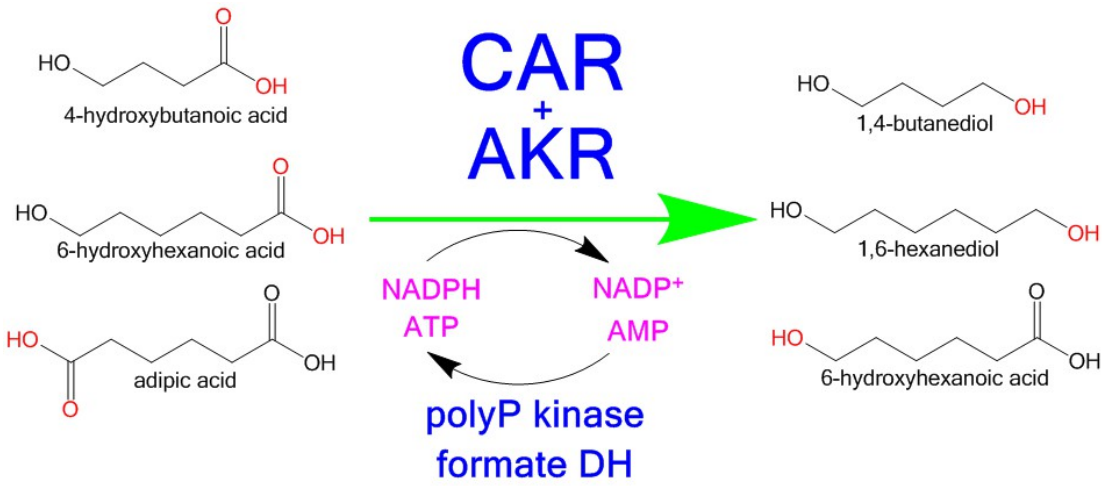


Figure 1: Carboxylic acid reductases (CAR) reduce carboxylic acids to aldehydes using ATP and NADPH as cofactors. In this work, we demonstrated that 12 bacterial CARs can reduce a broad range of bifunctional carboxylic acids. Several CARs catalyzed a high conversion of 4-hydroxybutanoic and adipic acids to 1,4-butanediol, 1,6-hexanediol, and 6-hydroxyhexanoic acid in vitro *(in combination with cofactor regenerating systems and aldo-keto reductases) and* in vivo *(in E. coli cells).*

Taeho Kim
Ph.D. Candidate

B.S., 2009, Seoul National University
M.S., 2011, Seoul National University

Supervisor: Alexander Yakunin
Co-Supervisor: Radhakrishnan Mahadevan

Engineering, screening and characterization of enzymes for metabolic engineering for the production of high-value chemicals or biofuels

Development of sustainable energy and chemical generation is a crucial step towards solutions for global challenges including alleviation of climate changes and dependence on fossil fuels. Photosynthetic chemical and biofuel synthesis in cyanobacteria has emerged as a promising biotechnology for sustainable production of value-added compounds due to several advantages for bio-industrial processes, such as CO_2 mitigation, simple input requirements, rapid and simple genetics, and carbon-neutral applications. In this study, a target cyanobacterium strain, *Synechococcus elongatus* PCC 7942, is engineered to produce 1,3-butanediol (1,3BDO), whose biosynthesis in cyanobacteria has never been demonstrated. 1,3BDO is a direct precursor to catalytically synthesize 1,3-butadiene, which is used in the manufacture of synthetic rubber and latex. For cyanobacterial synthesis of 1,3BDO, novel pathways are proposed in this study that are advantageous in the cyanobacterial system in terms of driving force in metabolism and cofactor requirement. The proposed 1,3BDO pathway utilizes pyruvate as a starting metabolite and consists of three enzymes: pyruvate decarboxylase, aldolase, and aldo-keto reductase. Introduction of the heterologous 1,3BDO pathway genes was conducted with the target cyanobacterium strain. Also, the platform of cyanobacteria engineering is established to be further used in cyanobacteria engineering applications. A novel method of product detection as well as characterization of mutant cyanobacteria strains will be developed and used for successful demonstration of the unprecedented photosynthetic bioconversion of CO_2 into 1,3BDO.

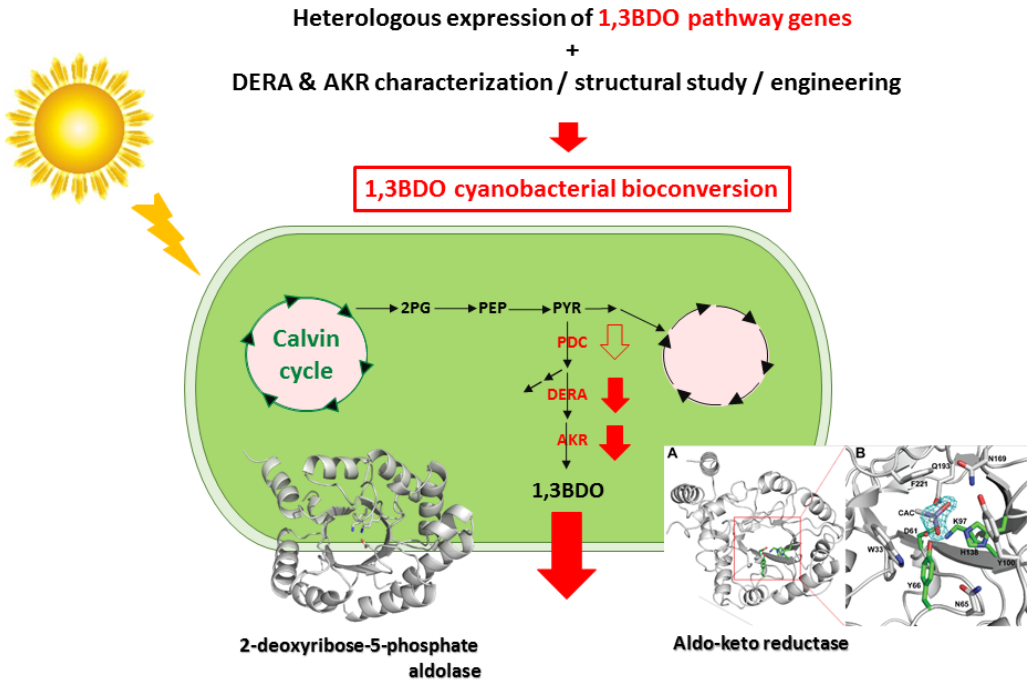


Figure 1: Heterologous expression of 1,3BDO pathway genes.

Research Highlights

T. Kim *et al.* (2017). Novel aldo-keto reductases for the biocatalytic conversion of 3- hydroxybutanal to 1,3-butanediol: Structural and biochemical studies. *Appl. Environ. Microbiol.*, **83**.

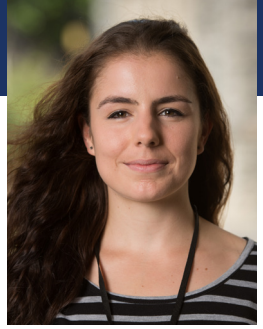
J. C. Joo *et al.* (2017). Alkene hydrogenation activity of enoate reductases for an environmentally benign biosynthesis of adipic acid. *Chemical Science*, **8**, 1406-1413.

J. C. Joo *et al.* "Biocatalytic production of adipic acid from unsaturated six-carbon dicarboxylic acids using 2-enoate reductases", oral presentation at the *Gordon Research Conference, Biocatalysis*. Smithfield, Rhode Island. July 6-11, 2014.

Research Highlights

S. Kraus, E. A. Edwards. "Aerobic and anaerobic degradation of chlorinated anilines and chlorinated benzenes in a complex site in Latin America.", oral presentation at the *RemTEC Conference*. Denver, Colorado. March 7-9, 2017.

S. Kraus, E. A. Edwards. "Evaluation of potential inhibitory effects in complex contaminant mixtures", poster presentation at the *16th International Symposium on Microbial Ecology*. Montreal, QC. August 21-26, 2016.



Suzana Kraus
M.A.Sc. Candidate

B.Eng., 2014, Universidade Estadual Paulista "Julio de Mesquita Filho"

Supervisor: Elizabeth Edwards

Natural and enhanced aerobic and anaerobic degradation of dichlorobenzene (DCB), chloroaniline (CA), dichloroaniline (DCA) and dichloronitrobenze (DCNB)

This research is part of a larger research program that aims to identify the best remediation technique at a highly contaminated industrial site in Brazil. Among various techniques that are being studied, this one aims to evaluate natural and enhanced aerobic and anaerobic degradation processes using groundwater and soil samples. This site has over 30 different compounds in its plume, but the compounds of interest for this research are mainly dichlorobenzene (DCB), chloroaniline (CA), dichloroaniline (DCA) and dicholoronitrobenze (DCNB).

To do so, soil and groundwater samples were shipped from Brazil to Canada in 2015 in order to start the experiments in the BioZone Lab. Due to this complex mixture of contaminants at the site, 92 different microcosms had to be set up, according to the divisions below:

For the aerobic ones, the bottles were divided into:

a. Sterile controls: with sodium azide and mercuric chloride;
b. Active controls: no amendments;
c. Vitamin amended: added some vitamins, N and P.

For the anaerobic microcosms, they were set up as follows:

a. Sterile controls: with sodium azide and mercuric chloride;
b. Active controls: no amendments;
c. Donor amendment: ethanol and lactate were added, 100 mg/L each;
d. Sulfate amendment: 2mM sulfate added;
e. Nitrate amendment: 2mM nitrate added.

After that, the microcosms were analyzed in both HPLC and GC to understand how the contaminants were behaving in each of the above-mentioned situations. Also, some qPCR analysis were performed in some of the bottles.

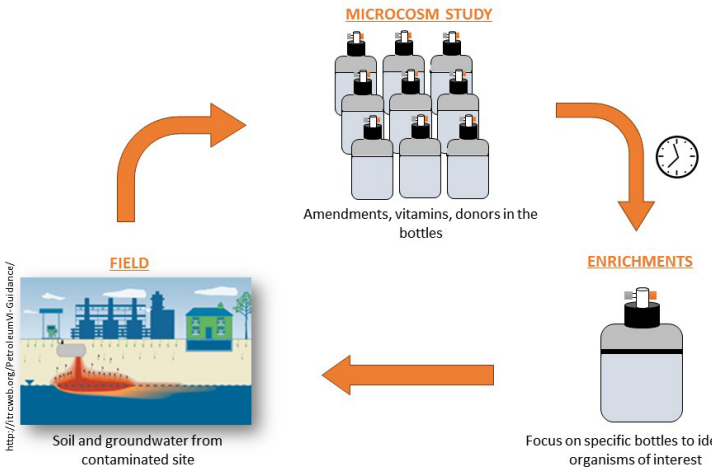


Figure 1: Soil and groundwater samples from a contaminated industrial site in Latin America were used in a microcosm study with different amendments and under different conditions (aerobic and anaerobic).

B.Tech., 2008, Tamil Nadu Agricultural University
M.Tech., 2010, Tamil Nadu Agricultural University
Ph.D., 2015, McGill University

Kiruba Krishnaswamy
Postdoctoral Fellow

Supervisor: Levente Diosady

K. Krishnaswamy, L. Diosady. "Food fortification: An effective approach to improve global health", oral presentation at the *Engineering World Health Symposium*. Toronto, ON. February 22, 2016.

Microencapsulation of iron premix in double-fortified salt (DFS) and quadruple fortified salt (QFS)

Goals: Iron deficiency affects approximately 1.6 billion people worldwide, and India is one of the countries worst affected by anemia. The goal of the program is to distribute Double-Fortified Salt (DFS) with iron and iodine in an efficient, transparent manner utilizing the public distribution system across Uttar Pradesh, to reduce iron deficiency anemia and iodine deficiency among the bottom economic quintile comprising of children and women of child bearing age reaching nearly 15 million people.

Key Objectives:

- Refining, transferring and scale-up technology for double fortification of salt (DFS)
- Facilitating its large scale application through the Public Distribution System in Uttar Pradesh State, India

Progress towards Results:

- The microencapsulation technology for DFS has been refined and updated. Two different approaches, namely extrusion method and spray drying method, were studied to examine the efficiency of encapsulated iron.
- The extrusion method for making iron premix contains ferrous fumarate (iron source) and semolina as binder in the core at 80:20 ratio, followed by colour masking with titanium dioxide (25%) and finally coating with soy stearin (fat coat) to prevent the interaction of iron with iodine and moisture in DFS.
- Based on the previous field test in India, it was observed that the iron premix in DFS tends to float in water, which might be due to the fat overcoat. In order to increase the wetting properties of the iron premix, lecithin at 0.5%, 1%, 1.5% was added to soy stearin at 10%, 15% and 20% concentration.
- It was found that the 20% soy stearin and 1% lecithin combination provided better wetting properties to iron premix, with colour values (L,a,b) close to that of natural salt.

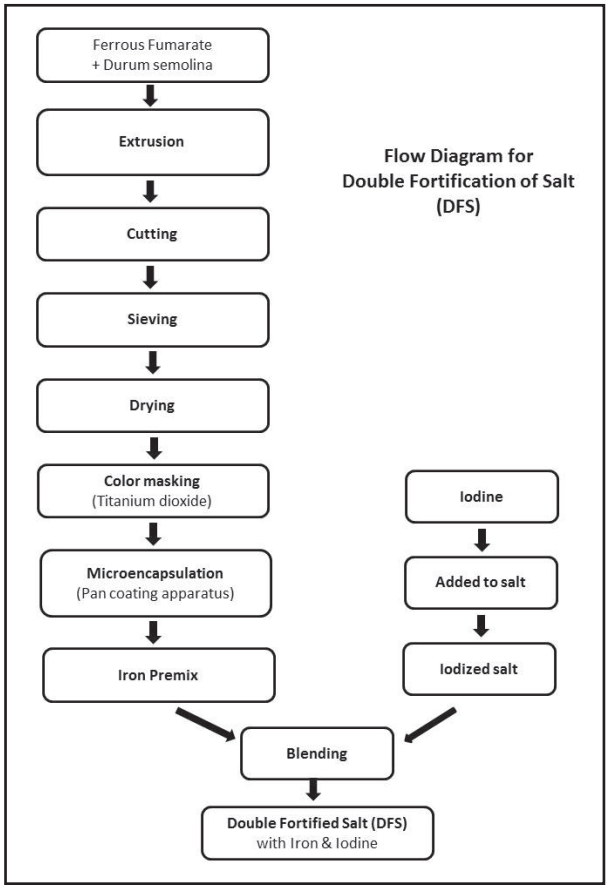


Figure 1: Flow diagram for double fortification of salt.

Research Highlights

T. V. Vuong, M. Foumani, B. MacCormick, R. Kwan, E. R. Master. (2016). Direct comparison of gluco-oligosaccharide oxidase variants and glucose oxidase: Substrate range and H2O2 stability. *Sci. Rep.,* **6**.

R. Kwan, T. V. Vuong, M. Foumani, B. MacCormick, E. R. Master. "Increasing oligosaccharide oxidase (AA7) stability and substrate range through protein engineering and discovery", poster presentation at the *Annual General Meeting of the Industrial Biotechnology Network*. Montreal, QC. May 18-19, 2016.

NSERC Canada Graduate Scholarships - Master's Program, NSERC. (2016)

Rachel Kwan
M.A.Sc. Candidate

B.Sc., 2015, University of Alberta

Supervisor: Emma Master

In vitro synthesis and screening of carbohydrate oxidases

Oligosaccharide oxidases from the Auxiliary Activity family 7 (AA7; www.cazy.org) have been proposed for varied industrial applications, ranging from alternatives to glucose oxidase (GO) in baking applications, to synthesis of bifunctional macromolecules used as bio-based cross-linking molecules. In order to expand the range of available carbohydrate oxidases with different substrate profiles, additional AA7 enzymes will be selected from uncharacterized subfamilies identified through phylogenetic analysis of the AA7 sequences. We are optimizing methods of *in vitro* protein synthesis in an effort to rapidly map the protein phylogeny. Resulting enzymes will be screened for activity through colorimetric detection of H_2O_2; the reduction potential of synthesized enzymes will also be measured and correlated to substrate preference. This approach will be used to identify clusters within the AA7 phylogeny that should be targeted for recombinant protein production and detailed characterization.

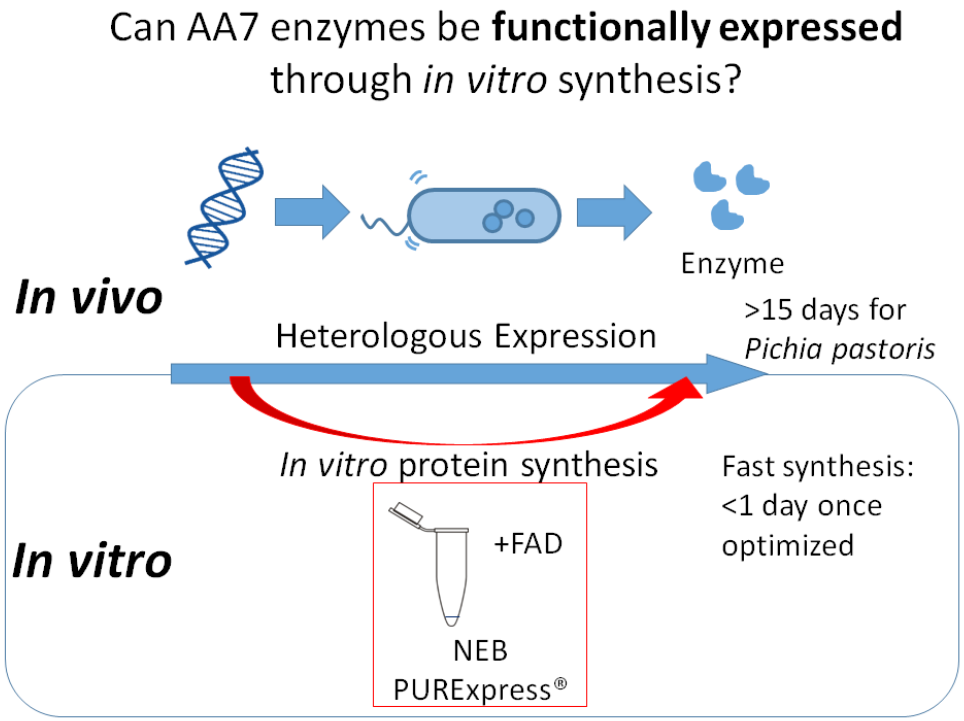


Figure 1: The cell-free in vitro *protein synthesis versus* in vivo *synthesis.*

Peter (HyunWoo) Lee
M.A.Sc. Candidate

B.A.Sc., 2014, University of British Columbia

Supervisor: Elizabeth Edwards

Characterization of the microbial community inside an anaerobic digester treating solid organic waste

Large portions of Canada's waste end up in landfills. Landfills account for 20% of methane emissions in Canada. Methane is a well-known greenhouse gas (GHG). A measure to decrease wastes diverted to landfill is needed. Anaerobic digestion has the ability to treat solid organic waste as well as produce biogas as a potential energy source.

Anaerobic digestion has been researched since the early 1920's. However, no research has been done on a sequentially-fed anaerobic digester inoculated with pulp mill sludge, treating solid organic waste. We need a better understanding of the microbial community inside the system and how the microbial community reacts to different feed compositions.

Leachate and digestate samples are collected periodically to monitor the change in microbial community. DNA are extracted for sequencing and quantification through qPCR. The data will help us correlate the behaviours of the microbial community to changing feed compositions. Furthermore, we can better predict changes in microbial community to stabilize the anaerobic digestion system and enhance system performance. This research can be applied in the waste industry to improve efficiency of anaerobic digestion systems.

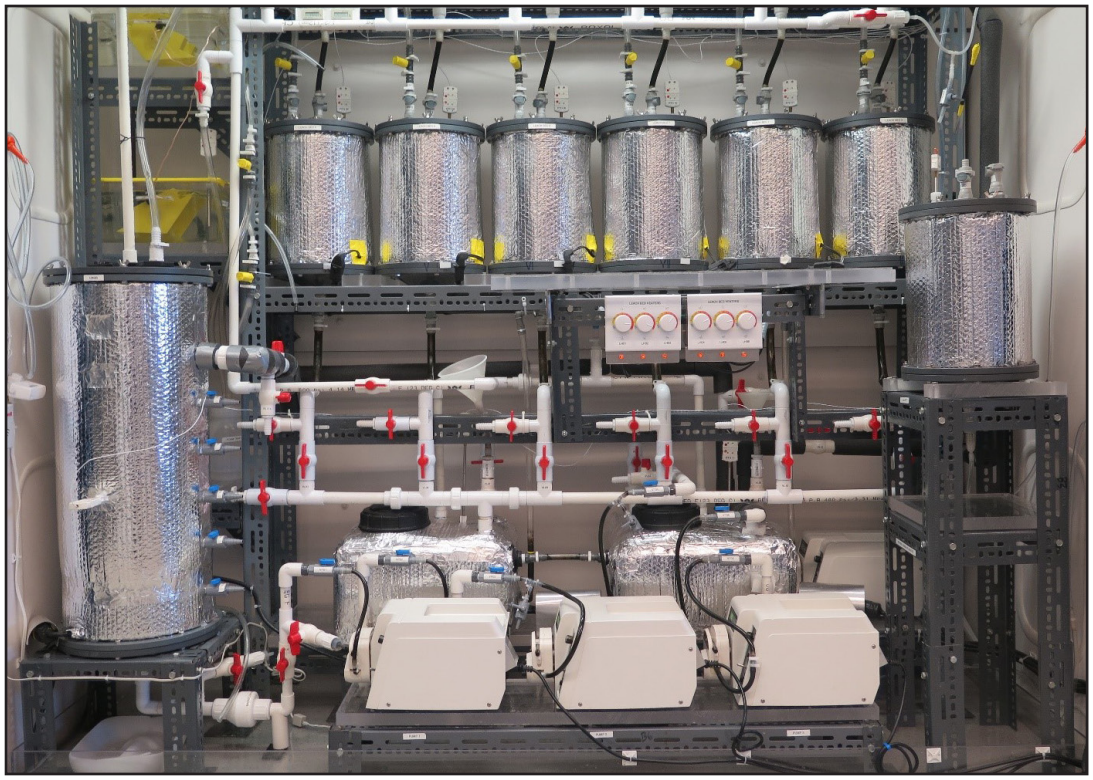


Figure 1: Daisy the Digester.

Research Highlights

H. Lee, N. G. H. Guilford, E. A. Edwards. "Characterization of the microbial community of an anaerobic digester treating solid waste", poster presentation at the *International Society for Microbial Ecology*. Montreal, QC. August 25, 2016.

Research Highlights

N. Beloglazova *et al.* (2015). CRISPR RNA binding and DNA target recognition by purified Cascade complexes from *Escherichia coli*. *Nucleic Acids Res.*, **43**, 530-543.

S. Lemak *et al.* (2014). The CRISPR-associated Cas4 protein Pcal 0546 from *Pyrobaculum calidifontis* contains a [2Fe-2S] cluster: Crystal structure and nuclease activity. *Nucleic Acids Res.*, **42**, 11144-11155.

S. Lemak *et al.* (2013). Toroidal structure and DNA cleavage by the CRISPR-associated [4Fe-4S] cluster containing Cas4 nuclease SSO0001 from *Sulfolobus solfataricus*. *J. Am. Chem. Soc.*, **135**, 17476-17487.

S. Lemak *et al.* (2012). Structure and activity of the cold-active and anion-activated carboxyl esterase OLEI01171 from the oil-degrading marine bacterium *Oleispira antarctica*. *Biochem. J.*, **445**, 193-203.

Sofia Lemak
Ph.D. Candidate

B.A.Sc., 2011, University of Western Ontario
M.A.Sc., 2013, University of Toronto

Supervisor: Alexander Yakunin

Characterization and engineering of the RNA-guided Cascade complex from *Escherichia coli*

The Clustered Short Palindromic Repeats (CRISPR) immunity system utilizes a wide range of effector complexes in order to target and destroy invading nucleic acids. In the Type I systems, all are similar multi-subunit complexes, with varying composition. The CRISPR-associated complex for antiviral defense (Cascade) from the Type I-E system of *Escherichia coli* has been shown to be a versatile tool for bacteria to protect themselves against invading generic elements during CRISPR immunity.

Type I is the most abundant CRISPR type in all organisms studied and several Type I Cascade complexes have shown versatility in complex composition. While the structure of the *E. coli* Cascade has been solved, the precise mechanism of action is not completely understood. Working both in the Interference and Adaptation stages of immunity, the role of Cascade has been shown to vary, and the specifics of the interactions between protein, RNA and DNA have not been described. By analyzing the proteins and substrate sequences in their native state we can eliminate unnecessary components and create a more efficient complex.

In order to better understand the specificity and flexibility of the Cascade complex, my project goal is to characterize both the crRNA and protein components of the *E. coli* Cascade with respect to assembly and function. Elucidating the minimal requirements for each of these components that are still able to efficiently bind target DNA will lead to a clearer understanding of the mechanisms involved as well as the creation of a more flexible system that can be applied to several aspects of biology.

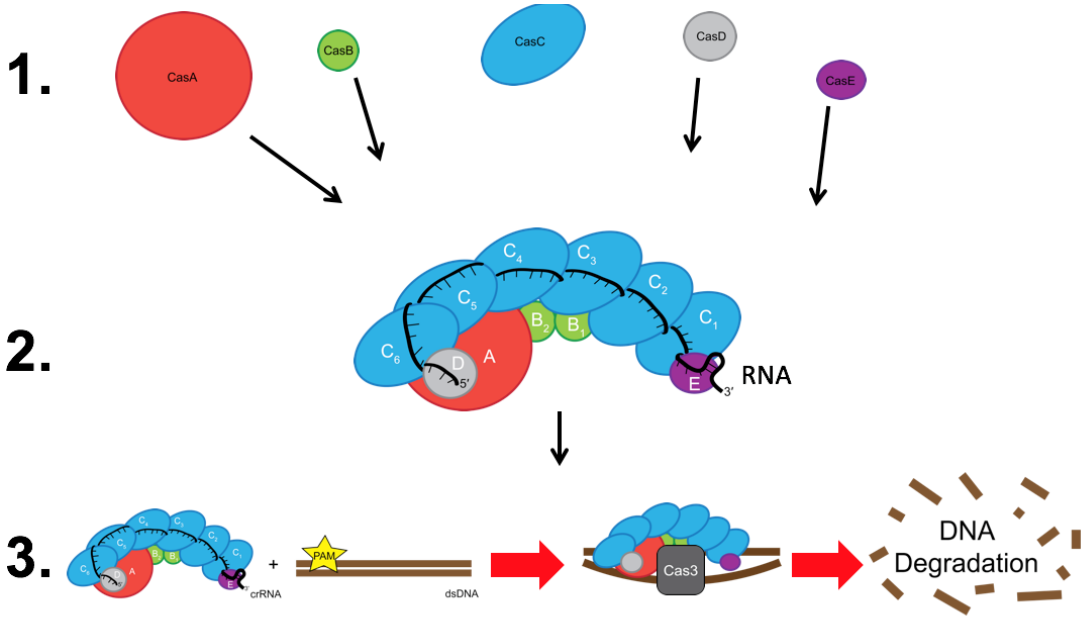


Figure 1: Composition (1), organization (2), and function (3) of the E.coli Cascade complex.

Line Lomheim
Laboratory Technician

M.Sc., 1997, Norwegian University of Science and Technology
M.A.Sc., 2002, University of Toronto

Supervisor: Elizabeth Edwards

Microcosm studies to assess *in situ* biodegradation rates

As a laboratory technician I work on various research projects and provide assistance with equipment and procedures to students and laboratory staff. Some of the techniques that I teach are gas and liquid chromatography, preparation of standards, calibration of analytical instruments, accuracy of measurements, use of syringes, techniques involved in microbial growth and maintenance of anaerobic microorganisms, characterization of microbial communities in enrichment cultures, groundwater and soil samples (qPCR, Pyrotag and MiSeq sequencing) and the creation of anaerobic microcosms. I also provide support to collaborating laboratories.

With a background in environmental technology and contaminant hydrogeology, and work experience as an environmental consultant, I have taken on projects with industrial partners. I conduct treatability studies to assess bioremediation options for organic pollutants at contaminated sites. In these studies we work with compounds such as chlorinated hydrocarbons, herbicides, pesticides and chlorinated aromatics used in pesticide production. We create microcosms using soil and groundwater from the contaminated site (see photo). We assess intrinsic biodegradation in the groundwater, evaluate ways to improve degradation efficiency through various amendments, and evaluate whether bioaugmentation with microbial consortia is needed. Collaborations with other universities and industry partners involve the use of chemical reduction, such as zero-valent iron, in combination with biodegradation as remediation technologies.

Figure 1: Anaerobic microcosms created from soil and groundwater from a contaminated site.

Research Highlights

C. M. D. Kocur *et al.* (2016). Long-term field study of microbial community and dechlorinating activity following carboxymethyl cellulose-stabilized nanoscale zero-valent iron injection. *Environ. Sci. Technol.,* **50**, 7658-7670.

C. M. D. Kocur *et al.* (2015). Contributions of abiotic and biotic dechlorination following carboxymethyl cellulose stabilized nanoscale zero valent iron injection. *Environ. Sci. Technol.,* **49**, 8648-8656.

L. Lomheim *et al.* "Microbial population changes during degradation of chlorinated compounds at an nZVI field trial", oral presentation at the *9th International Conference on Remediation of Chlorinated and Recalcitrant Compounds*. Monterey, California. May 21, 2014.

Zheng Lu
M.Eng. Candidate

B.Sc. (Hons), 2015, University of Saskatchewan

Supervisor: D. Grant Allen
Co-Supervisor: Yaldah Azimi

Enzyme application on biosludge

Wastes are generated daily from various sources, including agriculture, industry and households. These waste will eventually turn into biosludge with the enrichment of microorganisms. Disposing the biosludge is expensive. The traditional biosludge treatment of land filling or incineration is still considered to be not very effective. Anaerobic digestion was developed as a better alternative for the biosludge treatment as it can reduce the solid content and, at the same time, harvest biogas that is mainly composed of methane as a renewable energy. The overall process of anaerobic digestion consists of disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis. However, the retention time for the whole process is very long due to that the process of hydrolysis limits the overall rate of the anaerobic digestion. It is because most biomass substrates are in the polymeric form, which cannot be readily used by the microorganisms. During hydrolysis, macromolecules are degraded by the hydrolytic enzymes that are released from the microbial cells, which then are fermentable for these organisms. The methods of thermal, mechanical, chemical, enzymatic, sonic biosludge pre-treatment have been developed to show that they can assist the hydrolysis process to various degrees. However, we will focus on using the exogenous enzymes to enhance the reaction rate.

There are many advantages of using external enzymes in the treatment. Enzymes will not be interfered by microorganisms, predators, and inhibitors of microbial metabolism. However, the cost of using commercial enzymes is high. To reduce the cost, we are proposing using the enzymes from the fruit waste into the treatment. Enzymes from fruit waste are cheaper, which has the potential to be economically useful in large-scale and more readily available. Reuse of waste would be more environmentally friendly. Fruit wastes contain a variety of enzyme activities capable of numerous catalytic functions for them to be useful in heterogeneous substrates like sludge biomass. Specifically, our research goal is to apply crude extracted enzymes from the fruit waste of kiwi, pineapple and papayas, which are known to have proteolytic functions, onto the different kinds of sludges. And then, we will see how that affects hydrolysis and biogas production.

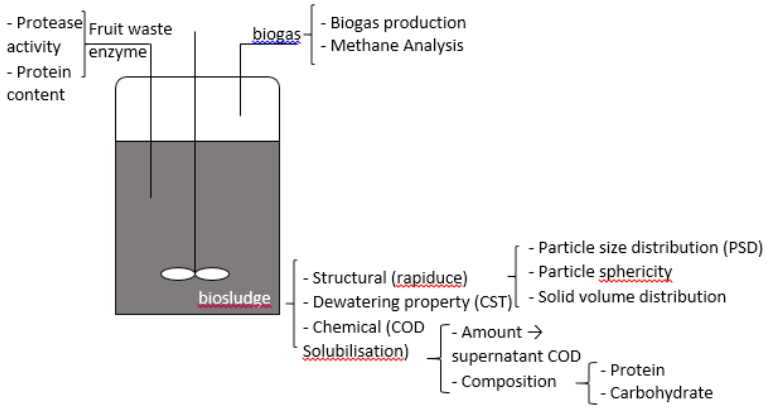


Figure 1: The enzymatic effects of fruit waste (kiwi, pineapple and papayas) on sludge hydrolysis in anaerobic digestion will be examined on three different parts: fruit waste enzymes, biosludge and biogas. The total protein content in the fruit waste and the activities of the hydrolytic enzymes will first be tested. The effects of enzymes on the biosludge and biogas will be observed. For changes in biogas, biogas production and methane analysis will be tested. The structural changes, COD solubilisation and dewatering property of biosludge will also be examined.

Fei Luo
Postdoctoral Fellow

B.Eng., 2008, Nanjing University of Technology
M.Sc., 2009, Nanyang Technological University
Ph.D., 2016, University of Toronto

Supervisor: Elizabeth Edwards

Anaerobic BTEX biodegradation

Benzene is a prevalent contaminant in the environment as a result of inadvertent leaks or spills of petroleum products. The presence of benzene in groundwater is of particular concern because it is a known human carcinogen and, therefore, its concentration in drinking water is regulated to a few parts per million. Groundwater impacted by petroleum hydrocarbon contamination typically becomes anaerobic. My overall goal is to understand the anaerobic biodegradation process of benzene in groundwater environments, and to transfer novel bioremediation technology to industry.

Over the past 20 years, microbial cultures have been enriched that are capable of degrading benzene in the absence of oxygen. During my previous Ph.D. work, the key organisms in two benzene-degrading enrichment cultures have been successfully identified. These organisms belong to relatively restricted phylogenetic groups, and hence their 16S rRNA gene sequences could be potentially used as molecular biomarkers indicative of *in situ* biodegradation activity at contaminated sites. Although the organisms have been identified, the functional genes and pathways of anaerobic benzene activation remain elusive. As a PDF, I am working on characterizing the benzene activation genes using metagenome and metaproteome approaches.

Also, I am working on developing biomarkers from the characterized 16S rRNA sequences of currently known anaerobic benzene degraders. One challenge of this work is that there is currently scarce evidence linking the presence of benzene-degrading organisms and biodegradation potential. I aim to establish clear links between benzene-degrading activity and the presence of microbes known to degrade benzene anaerobically by tracking designed molecular markers in benzene contaminated soil samples, and correlate the emergence of these markers to benzene degradation activity. The designed and verified biomarkers can be used to confirm and monitor *in situ* biodegradation of benzene. Also, I am working towards bringing an enriched benzene-degrading culture to industrial scale and performing a pilot scale study to verify bioaugmentation as a novel tool for *in situ* benzene remediation.

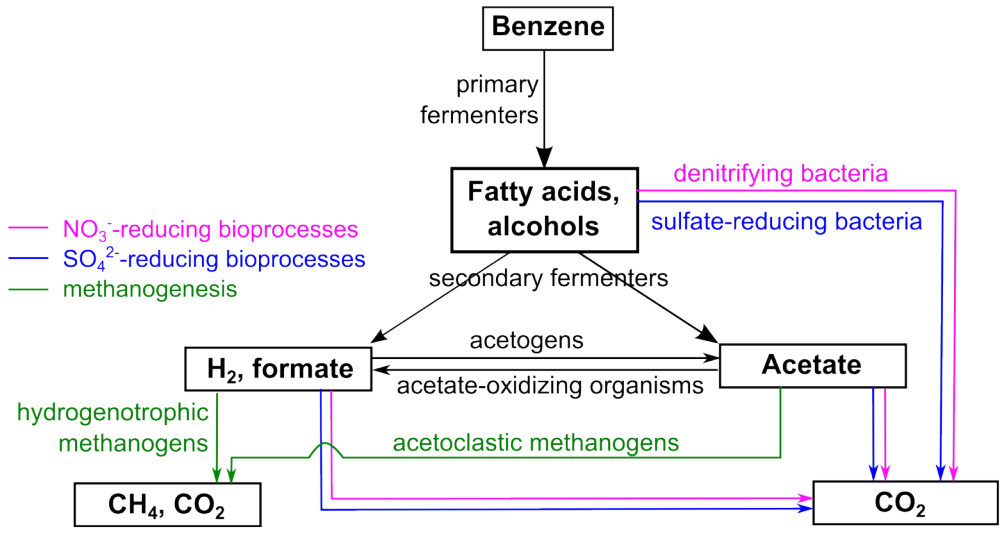


Figure 1: Syntrophic microbial community in anaerobic benzene-degrading cultures.

Research Highlights

W. Zhai *et al.* (2017). Arsenic methylation and its relationship to abundance and diversity of arsM genes in composting manure. *Sci. Rep.,* **7**.

F. Luo, C. E. Devine, E. A. Edwards. (2016). Cultivating microbial dark matter in benzene-degrading methanogenic consortia. *Environ. Microbiol.,* **18**, 2923-2936.

F. Luo *et al.* (2014). Metatranscriptome of an anaerobic benzene-degrading, nitrate-reducing enrichment culture reveals involvement of carboxylation in benzene ring activation. *Appl. Environ. Microbiol.,* **80**, 4095-4107.

Mitacs Accelerate Fellowship , MITACS. (2016-2017)

Student Paper Award, GeoSyntec. (2015)

BioZone Graduate Scholarship, BioZone. (2014)

Research Highlights

T. V. Vuong, M. Foumani, B. MacCormick, R. Kwan, E. R. Master. (2016). Direct comparison of gluco-oligosaccharide oxidase variants and glucose oxidase: Substrate range and H2O2 stability. *Sci. Rep.,* **6**.

B. MacCormick, E. R. Master. "Applications of gluco-oligosaccharide oxidases for carbohydrate modification", oral presentation at the *5th International Conference on Biorefinery and Bioenergy*. Vancouver, BC. August 10-12, 2016.

M. Foumani, T. V. Vuong, B. MacCormick, E. R. Master. (2015). Enhanced polysaccharide binding and activity on linear β-glucans through addition of carbohydrate-binding modules to either terminus of a glucooligosaccharide oxidase. *PLoS One,* **10**.

Ben MacCormick
Research Assistant

B.Sc., 2014, University of Waterloo
M.A.Sc., 2016, University of Toronto

Supervisor: Emma Master

Applications of gluco-oligosaccharide oxidase

The vast intrinsic value of lignocellulosic biomass is beginning to be unlocked thanks to innovative biorefinery techniques, generating high quality process streams of the constituent cellulose, hemicellulose, and lignin fractions. These renewable products have the potential to displace a variety of petroleum-based fuels, materials, and fine chemicals. While there are many established applications for cellulose, such as textiles, pulp and paper, and biofuel production, less attention has been given to hemicellulose utilization, despite it comprising approximately one-third of dry weight in most plant material.

A significant barrier to the development of hemicellulose-based products is its wide range of structural and chemical variability between plant species. Whereas cellulose is a simple linear homopolymer of glucose units, hemicellulose can consist of several 5 and 6 carbon sugars with significant branching, acetylation, and covalent linkages to other cell wall components. However, this diversity of chemical functionalities could be exploited to allow for targeted modification of hemicellulose fragments. To achieve such transformations, the growing toolkit of carbohydrate active enzymes (CAZymes) provides an excellent resource for the introduction of reactive chemical groups to carbohydrate structures in a highly specific, mild, and efficient manner.

My current research focuses on generating novel materials from oligosaccharides released during the hydrolysis of glucuronoxylan and galactoglucomannan; hemicelluloses derived from hardwood and softwood, respectively. Using both site-specific enzymatic oxidation and chemical coupling, functionalized monomers can be constructed which are capable of reacting to form linear or cross-linked polymers with potentially useful properties such as biodegradability, low oxygen permeability, metal ion chelation, and interaction with cellulose. Such a material should also be generated using environmentally benign techniques whenever possible; to this end, processes must be optimized to reduce the use of organic solvents, maintain high atom economy, and proceed at mild to moderate temperatures. The successful development of value-added technologies making use of hemicellulose would provide additional revenue streams in lignocellulosic biorefinery, and help renewable biomass reach new markets in the specialized polymer and materials sector.

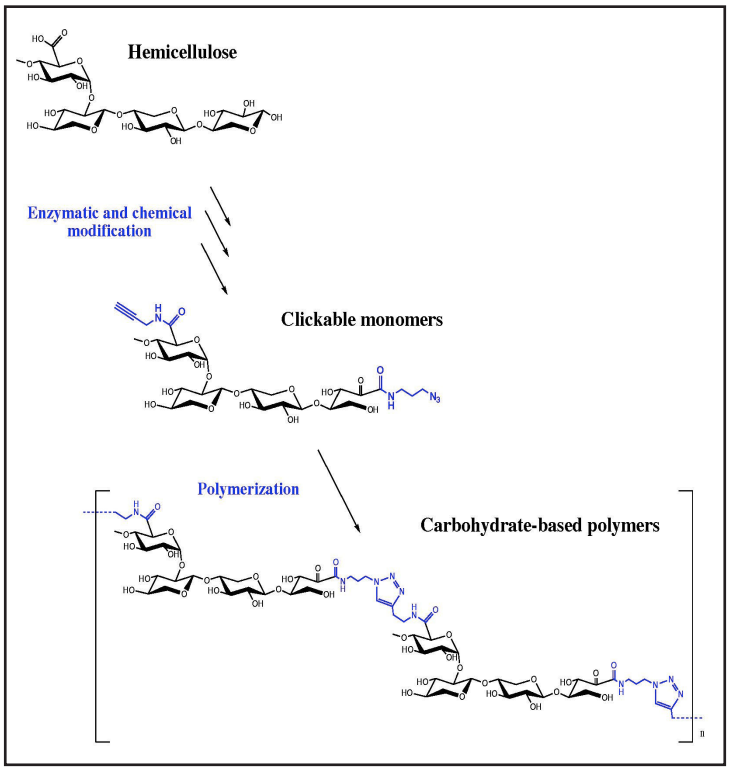


Figure 1: Modification of hemicellulose.

Elisse Magnuson
M.Sc. Candidate

B.Sc., 2016, University of Toronto

Supervisor: Elizabeth Edwards

Bioremediation of benzene

Benzene is a common contaminant of soil and groundwater, and is a highly toxic and persistent carcinogen. As such, remediation of benzene from contaminated sites and groundwater, particularly in or near sources of drinking water, is of great concern for public and environmental health. Benzene degradation under aerobic conditions occurs readily via microbial consumption, and this process is already well characterized. However, groundwater and other environments contaminated with benzene are often anaerobic or become anaerobic as oxygen is depleted rapidly through aerobic degradation. Degradation of benzene by microbial communities under anaerobic conditions is a slow and relatively uncharacterized process. It can occur naturally under different electron acceptor conditions, such as methanogenic, nitrate-reducing, and iron-reducing. My research aims to investigate benzene degradation by bacterial communities under nitrate-reducing conditions by studying the functional characterization of community members and through analysis of metabolite exchange. This will help to identify the key community members and their roles in benzene attenuation. A better understanding of this process is important to optimizing the commercial opportunities for benzene-degrading bacterial culture in hazardous site remediation.

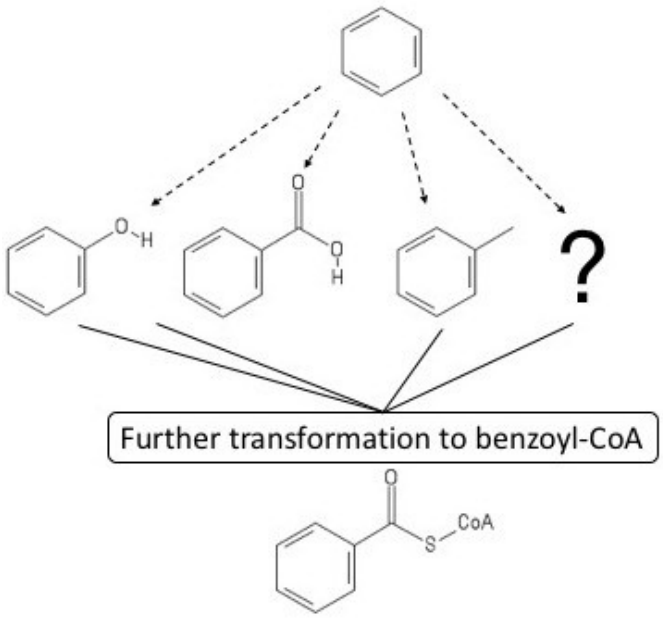


Figure 1: The initial step in the benzene degradation pathway is currently unknown, though there are several theories as to what the possible intermediates may be.

Research Highlights

E. Magnuson, F. Luo, E. A. Edwards. "Microbial community dynamics in nitrate-reducing benzene-degrading cultures", poster presentation at the *International Society for Microbial Ecology Conference*. Montreal, QC. August 25-26, 2016.

Queen Elizabeth II Graduate Scholarship in Science & Technology, Government of Ontario & the University of Toronto. (2016)

William and Dorothy Palm Graduate Scholarship in Science and Technology, Women's College Research Institute. (2016)

Conference Grant, University of Toronto; School of Graduate Studies. (2016)

Research Highlights

R. Malekzai, E. R. Master. “Hydrophobin coatings for improved water barrier properties of cellulose packaging”, oral presentation at the *Ontario-Quebec Biotechnology Meeting*. Waterloo, ON. May 26, 2016.

Roman Malekzai
M.A.Sc. Candidate

B.Sc., 2015, McMaster University

Supervisor: Emma Master

Hydrophobin interactions with lignocellulose and effects on enzyme action

Hydrophobins are small, secreted proteins found in filamentous fungi. In solution, they readily assemble at interfaces (liquid-liquid or solid-liquid) and fungi utilize this in nature to lower the surface tension of water, alter the hydrophobicity of the environment, or protect spores from immune detection.

My study concentrates on the so-called “class I” hydrophobins, which are encoded by Basidiomycete fungi and assemble as stable rodlets at interfaces. My overall goal is to correlate HFB sequence and structure to affinity to the chemistry and structure of main lignocellulose fractions (i.e., cellulose, hemicelluloses, and lignins). Moreover, I will investigate the impact of HFB binding to lignocellulose on the activity of carbohydrate active enzymes (CAZy). It is anticipated that this study will uncover HFB candidates for use as bio-based coatings for cellulose fibre, stabilizers of hemicellulose-derived emulsions, and stabilizers of lignin nanoparticle suspensions. By investigating the impact of HFBs on CAZy activities, this study may also establish HFB tools to promote or deter enzymatic degradation of lignocellulose materials.

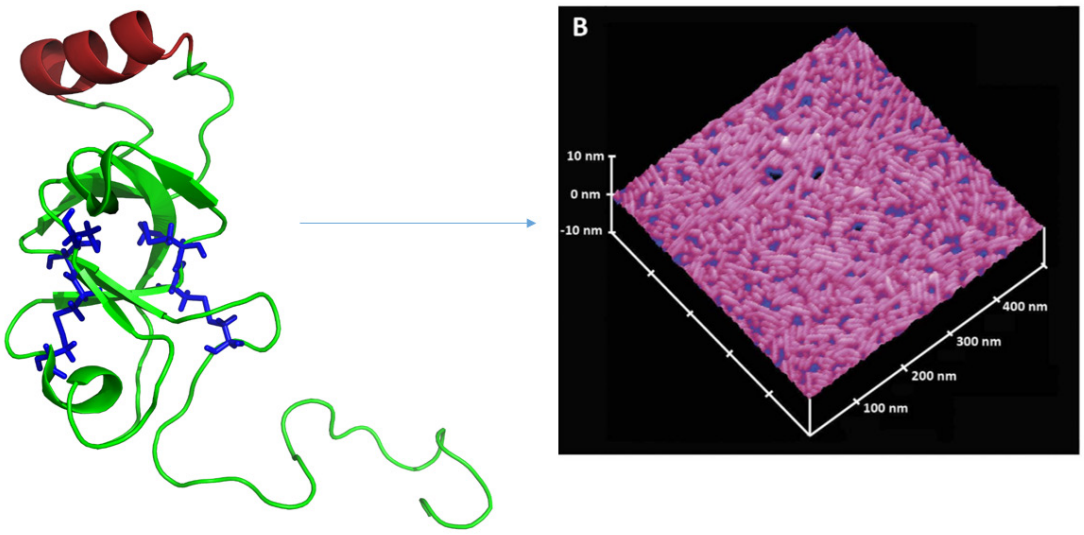


Figure 1: Class I hydrophobins assemble into oligomeric, chemically-resistant rodlets when exposed to an air-water interface. My research aims to determine the binding profile of 5 hydrophobins to lignin and cellulose before and after assembly.

Elisa McGee
Ph.D. Candidate

B.A.Sc., 2010, University of Toronto
M.A.Sc., 2012, University of Toronto

Supervisor: Levente Diosady

Fortification of tea with iron

The fortification of black tea with iron has the potential to reduce the prevalence of iron deficiency in the developing world. Tea is an ideal vehicle for food fortification because it is centrally processed, is the most consumed beverage globally (aside from water), and is consumed in regular quantities by those of all socioeconomic statuses in many regions. Unfortunately, there is a prominent technical difficulty because polyphenolic compounds present in tea, which are responsible for colour and flavour, form complexes with iron which reduce the bioavailability of both compounds and cause strong colour development. The objective of this study is to develop a fortification method such that these complexes do not form.

A spectrophotometric method was developed for the quantification of iron-polyphenol complex formation. Iron-polyphenol complex formation was further investigated with a variety of iron sources, temperatures, and polyphenol concentrations using a gallic acid model system and tea extract. Analysis and modeling of iron-polyphenol complex formation prompted the investigation of predicted inhibitors, specifically reducing and chelating agents. Many of these were tested in the presence of iron and black tea extract at pH levels relevant to brewed tea and iron absorption into the body. At the completion of this project, a process for effective iron fortification of tea based on our laboratory technique will be ready for *in vivo* evaluation of its effectiveness and pilot scale testing.

Figure 1: Fortification of tea with iron.

Research Highlights

E. J. T. McGee, A. R. Sangakkara, L. L. Diosady. (2017). Double fortification of salt with folic acid and iodine. *J. Food Eng.,* **198**, 72-80.

E. McGee, L. Diosady. "Fortification of tea with iron", oral presentation at the *International Conference on Food Chemistry and Hydrocolloids*. Toronto, ON. August 11, 2016.

E. McGee, L. Diosady. "Strategies for the fortification of tea with iron", poster presentation at the *WISE National Conference*. Toronto, ON. March 21, 2015.

E. McGee, L. Diosady. "Model system points to strategies for the fortification of tea with iron", oral presentation at the *International Conference on Food Chemistry and Technology*. San Francisco, California. November 17, 2015.

Ontario Graduate Scholarship, Government of Ontario & the University of Toronto. (2015, 2016)

Research Highlights

T. Meyer, X. Chen, H. N. Tran, D. G. Allen, E. A. Edwards. (2017). Natural Freezing-Thawing and Its Impact on Dewaterability and Anaerobic Digestibility of Biosludge. *Environ. Eng. Sci.,* **34**, 357-366.

T. Meyer, M. I. Yang, H. N. Tran, D. G. Allen, E. A. Edwards. (2016). Impact of resin and fatty acids on full-scale anaerobic treatment of pulp and paper mill effluents. *Environ. Eng. Sci.,* **33**, 394-403.

T. M. Fitamo, O. Dahl, E. Master, T. Meyer. (2016). Biochemical methane potential of kraft bleaching effluent and codigestion with other in-mill streams. *Tappi J.,* **15**, 80-88.

T. Meyer, E. A. Edwards. (2015). Corrigendum to "Anaerobic digestion of pulp and paper mill wastewater and sludge". *Water Res.,* **68**, 849.

T. Meyer, E. A. Edwards. (2014). Anaerobic digestion of pulp and paper mill wastewater and sludge. *Water Res.,* **65**, 321-349.

Torsten Meyer
Research Associate

Dipl. Ing., 2002, Technical University Berlin
Ph.D., 2008, University of Toronto

Supervisors: Elizabeth Edwards, D. Grant Allen
Co-Supervisor: Honghi Tran

Biomass handling in pulp and paper mills

Canadian pulp and paper mills generate 1.7 million tonnes (dry solids) per year of combined primary sludge and biosludge, and sludge handling often comprises more than half of the overall wastewater treatment costs at a mill. Currently, the most common methods of sludge disposal are landfilling and incineration, both of which are costly and energy intensive. In order for Canadian mills to stay globally competitive, and leave a smaller environmental footprint, solutions for a more effective handling of wastewater treatment sludge are needed. Part of my work involves developing, together with students, cost and energy effective dewatering methods such as freeze-thaw treatment, electro-dewatering, and adding mill waste to sludge. My research also aims at enhancing anaerobic digestion of pulp and paper mill wastewater and sludge. Anaerobic digestion of biosludge has the potential to save costs by decreasing the amount of sludge solids, increasing the ratio of primary sludge to biosludge and thereby improving the dewaterability of the sludge mix, and generating renewable energy in form of methane. The research includes laboratory experiments, and full-scale reactor monitoring studies in combination with multivariate data analyses. Besides wastewater treatment sludge, Canadian mills also generate about 27 million tons of black liquor dry solids per year, as a result of lignin removal during pulping. Black liquor is burned in recovery boilers to produce energy and recover the chemicals needed in the pulping process. My research also involves detecting the causes for poor recovery boiler performance by means of multivariate data analysis methods.

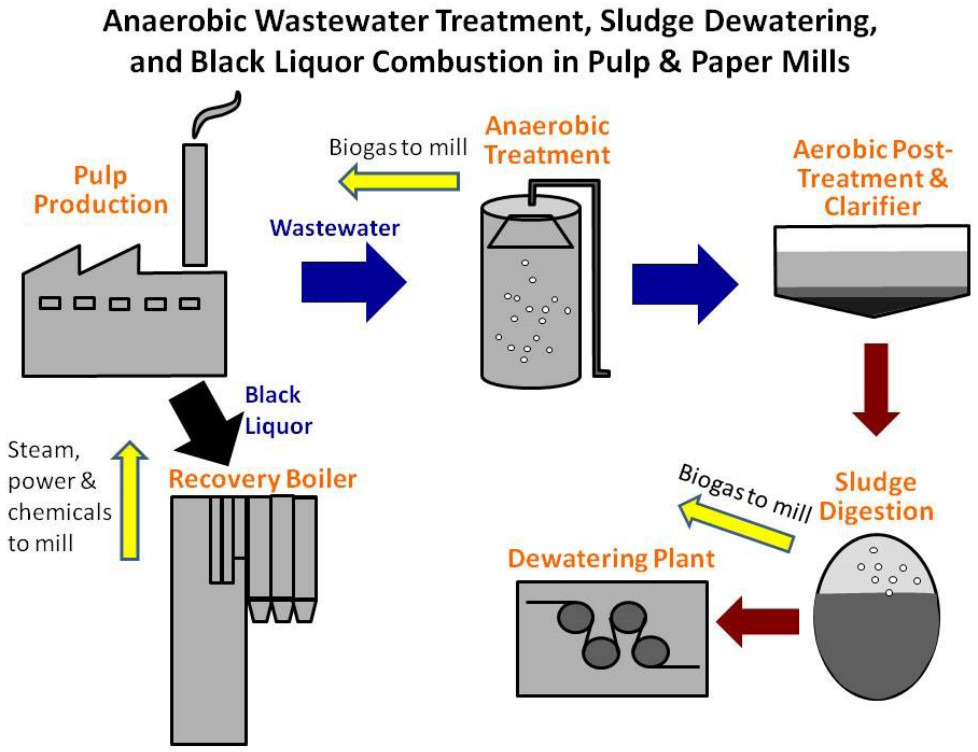


Figure 1: Anaerobic wastewater treatment, sludge dewatering, and black liquor combustion in pulp & paper mills.

Oluwasegun Modupe
Ph.D. Candidate

B.Sc., 2010, University of Ilorin
M.Sc., 2013, University of Ilorin
M.Sc., 2014, University College London

Supervisor: Levente Diosady

Quadruple fortification of salt

The world, especially the developing countries, is currently struggling with meeting up with the nutritional requirements of her growing populations; with micronutrients taking the central stage, given their roles in human growth, development, and functions. Fortification has been proposed as one of the ways of combating these micronutrient deficiencies. With the success made with iodized salt, it is becoming imperative to build other fortification models around iodized salt. Aside from the challenge of making the fortification of salt economical and acceptable, the nutrients in food fortified must be stable.

My research focuses on solving world micronutrient deficiency by adding some of the micronutrients with high-deficiency prevalence and irreversible devastating effect on humans into salt. The micronutrients of interest are iron, iodine, folic acid and vitamin B12. The chemistry of these micronutrients may cause interactions among the micronutrients. An effective and low-cost technology to prevent these interactions will be devised. The research will also focus on ways to reduce organoleptic effect the fortificants may have on salt. The prevention of interaction among the micronutrients and organoleptic effect will be ensured by microencapsulation. Reactive fortificants will be encapsulated while others will be dissolved in appropriate solvent, and sprayed on salt. The addition of the micronutrients is expected to have no big effect on the cost of salt.

Research Highlights

Paul Cadario Doctoral Fellowship in Global Engineering, University of Toronto; Faculty of Applied Science & Engineering. (2016)

Paul Cadario Doctoral Fellowship in Global Engineering, University of Toronto; Faculty of Applied Science & Engineering. (2017)

Research Highlights

C. M. D. Kocur *et al.* (2016). Long-term field study of microbial community and dechlorinating activity following carboxymethyl cellulose-stabilized nanoscale zero-valent iron injection. *Environ. Sci. Technol.,* **50**, 7658-7670.

O. Molenda, S. Tang, E. A. Edwards. (2016). Complete genome sequence of *Dehalococcoides mccartyi* strain WBC-2, capable of anaerobic reductive dechlorination of vinyl chloride. *Genome Announce.,* **4**.

O. Molenda, A. T. Quaile, E. A. Edwards. (2016). *Dehalogenimonas* sp. Strain WBC-2 genome and identification of its trans-dichloroethene reductive dehalogenase, TdrA. *Appl. Environ. Microbiol.,* **82**, 40-50.

K. Mayer-Blackwell *et al.* (2016). 1,2-DCA exposure alters the population structure, metabolism, and kinetics of a trichloroethene-dechlorinating *D. mccartyi* consortium. *Environ. Sci. Technol.,* **50**, 12187-12196.

Olivia Molenda
Ph.D. Candidate

B.Sc., 2011, York University

Supervisor: Elizabeth Edwards

Metagenomic investigation of *Dehalococcoides* used for bioremediation of chlorinated ethenes and ethanes in groundwater and soil

Chlorinated ethenes and ethanes are toxic, widespread and recalcitrant groundwater and soil contaminants. While currently there are strategies in place to reduce use and avoid release of these compounds, many sites remain contaminated due to historical military and industrial use. Bioaugmentation using a mixed microbial culture is an effective remediation strategy for these compounds. While the degradation pathways of certain contaminants such as perchloroethene, are already well established, further research is required for optimization of this strategy and for the discovery of novel pathways.

One of the most successful organisms capable of complete anaerobic reductive dechlorination, are the *Dehalococcoides mccartyi. D. mccartyi* have small genomes (~1.3Mbp) specialized for dechlorination each harbouring many different reductive dehalogenase genes. One of the more interesting features of their small streamlined genomes is the presence of high plasticity regions (HPRs) which contain multiple putative mobile elements, genomic islands, integration elements, prophages and show evidence of recombination. The HPRs account for the major differences between strains including their dechlorinating ability since the majority of reductive dehalogenases are found in HPRs. The mechanisms for recombination in HPRs are not yet well understood, and only a handful of reductive dehalogenases have been characterized to date.

Using a combination of metagenomic and amplicon sequencing, we cloned eight different strains of *D. mccartyi* from KB-1, a mixed microbial culture used for bioaugmentation. Combining bioinformatical analyses with molecular techniques such as qPCR, PCR and blue-native polyacrylamide gel electrophoresis/liquid chromatography/mass spectrometry, we discover new enzymes, new biological pathways and begin to shed light on gene transfer, recombination and invading pathogenic DNA in *D. mccartyi.* We hope results can be used to improve bioaugmentation strategies and broaden the range of compounds which can be degraded by *D. mccartyi.*

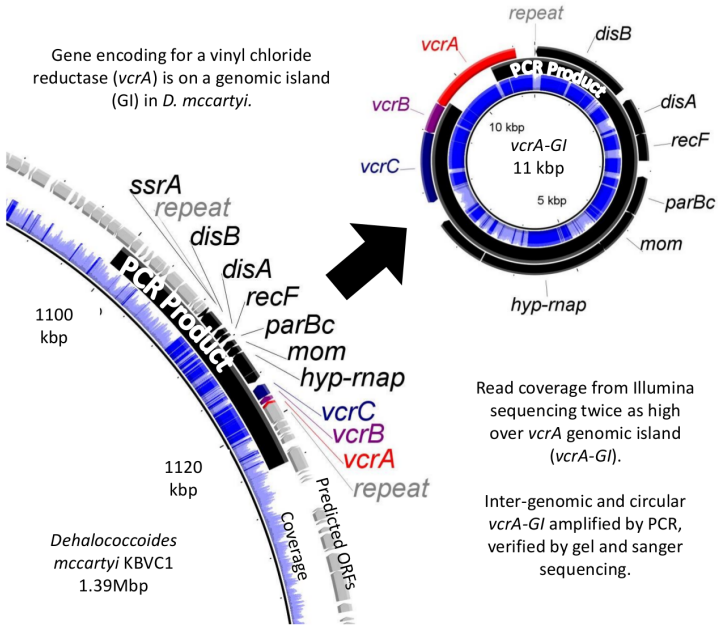


Figure 1: The vcrA-GI in Dehalococcoides mccartyi mobilizes via a circular intermediate.

Nadia Morson
M.Sc. Candidate

B.Sc., 2016, University of Guelph

Supervisor: Elizabeth Edwards

Horizontal gene transfer of the *vcrA*-genomic island of *Dehalococcoides mccartyi* in anaerobic dechlorinating cultures

Chlorinated solvents are common groundwater and soil contaminants, as a result of poor disposal practices from industry. Bioremediation provides a solution, as microorganisms can anaerobically metabolize these toxic compounds. Microorganisms, such as *Dehalococcoides mccartyi*, can degrade highly chlorinated compounds, such as perchloroethene and trichloroethene, to ethene, a non-toxic end-product.

Putative genomic islands (GI) have been identified using bioinformatics in *D. mccartyi*. One of these islands contains *vcrA*, a gene that encodes a reductive dehalogenase catalysing hydrogenolysis of vinyl chloride (VC) to ethene. It is unknown if and how these genomic islands are mobilized within microbial communities.

To observe horizontal gene transfer (HGT) among different strains of *D. mccartyi*, the KB-1 culture was mixed with Donna II to create a culture called "DKB". KB-1, a mixed microbial culture used for bioremediation, contains multiple strains of *D. mccartyi* which allows for complete degradation of chlorinated ethenes. Donna II, in collaboration with Cornell University, is a mixed microbial culture which contains only *D. mccartyi* strain 195.

The combination culture, DKB, was maintained in sub-cultures under different substrate conditions. The most selective pressure imposed on the DKB culture was feeding VC in the absence of ammonium in the medium. This is because the KB-1 *D. mccartyi* strain which contains the *vcrA*-GI can degrade VC, but cannot fix nitrogen. Whereas *D. mccartyi* strain 195 from Donna II, can fix nitrogen, but does not have the *vcrA*-GI and cannot degrade VC. Therefore, this condition favours a *D. mccartyi* strain 195 which has picked up the *vcrA*-GI from its environment, that allows it to degrade VC in the absence of ammonium.

One of the ways in which this HGT event can be determined is through development of *D. mccartyi* strain-specific biomarkers and bioinformatical analysis. This task is challenging in that most *D. mccartyi* strains have >98% 16S rRNA gene sequence similarity.

Determining *D. mccartyi* biomarkers will be a novel discovery, and will allow for the quantification and further study of this organism in mixed culture with possible in-field applications. Additionally, understanding the role of this *vcrA*-GI in HGT may provide better insight to the community evolution of these dechlorinating cultures, and open the possibility of naturally improving substrate range of *D. mccartyi* strains used for bioremediation.

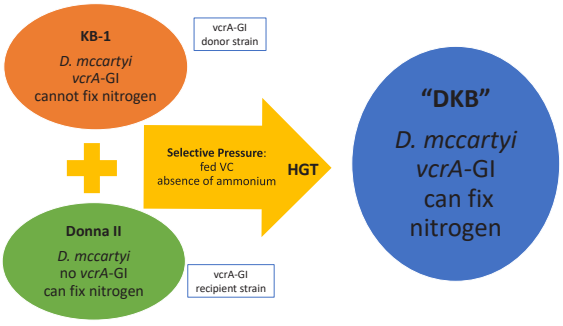


Figure 1: Creation of hybrid culture DKB from donors KB-1 and Donna II.

Research Highlights

N. Morson, E. Edwards. "Horizontal gene transfer of the vcrA-genomic island of *Dehalococcoides mccartyi* in anaerobic dechlorinating cultures", poster presentation at the *Second International Conference on Anaerobic Biological Dehalogenation*. Leipzig, Germany. March 26-29, 2017.

Research Highlights

F. Muhammad Razeq, E. Master. "Production and Characterization of a SGNH Hydrolase from Polysaccharide Utilization Loci Encoding Xylan-Active Enzymes", oral presentation at the *Gordon Research Seminar*. Andover, New Hampshire. July 2017.

F. Muhammad Razeq, E. Master. "Production and characterization of a protein with unknown function from polysaccharide utilization loci encoding xylan-active enzymes", poster presentation at the *NSERC Industrial Biocatalysis Network General Meeting*. Montreal, QC. May 18-19, 2016.

Ontario Graduate Scholarship, Government of Ontario. (2016)

NSERC Canada Graduate Scholarships - Master's Program, NSERC. (2015)

Helen L. Cross Memorial Graduate Scholarship, University of Toronto; Dept. of Chemical Engineering. (2015)

Fakhria Muhammad Razeq
M.Sc. Candidate

B.Sc., 2014, Carleton University

Supervisor: Emma Master

Biochemical characterization of new CAZymes from polysaccharide utilization loci and metagenome sequences

Hemicelluloses, including xylans and glucomannans, comprise a major fraction of underutilized lignocellulosic biomass, which have a potential for use in high-value biomaterials, such as bioplastics, adhesives, and various packaging materials. However, efficient utilization of hemicelluloses for production of biomaterials is hindered by the structural diversity and complexity of these biopolymers and often requires a large repertoire of different carbohydrate active enzymes (CAZymes).

The overall aim of my graduate thesis is to discover and characterize enzymes that are able to efficiently and selectively modify hemicelluloses, allowing us to fine-tune their chemistry, expand their utility and improve their performance in biomaterials. Specifically, I am looking at identifying and characterizing uncharted CAZymes from polysaccharide utilization loci (PULs) and metagenomic samples.

PULs are set of physically linked and functionally related genes that have evolved to work together to breakdown a specific polysaccharide (Fig. 1). While function of some of CAZymes on PULs are known or can be predicted, there are some proteins that have no known function. Discovery and characterization of these proteins of unknown function not only will help us to understand how all proteins on a given PUL work together, but also help us to find new CAZymes with novel functions that can be used for efficient utilization of plant polysaccharides.

Metagenomic sequences from anaerobic granules, beaver droppings and moose rumen microbial cultures enriched on cellulose and poplar hydrolysate available in our laboratory serve as another great source for discovery of new CAZymes with potentially novel activities. Together, PULs and metagenomic sequences, serve as a rich source for discovery and characterization of new and novel CAZymes, which can help to produce high-value, specialized biomaterials and chemicals from wood fibre and agricultural residues.

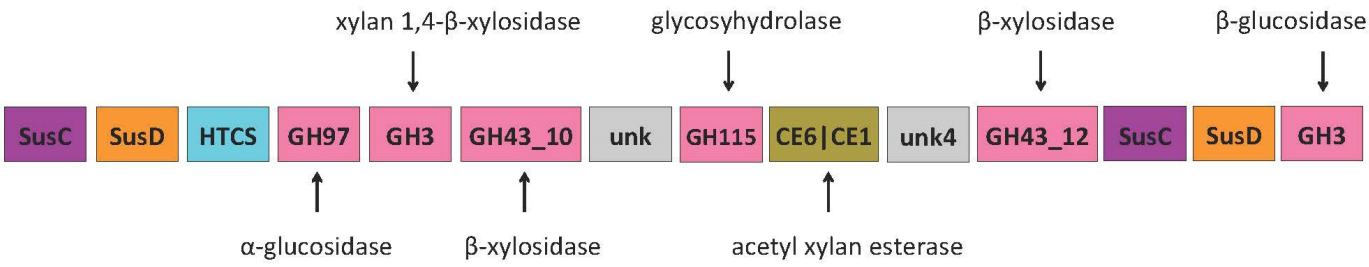


Figure 1: An example of a PUL showing possible functions of predicted CAZymes (as indicated by BLAST searches). Proteins of unknown function (unk) are in grey. Hybrid two-component system is responsible for PUL transcriptional activations and Starch utilization system C- and D-like protein (SusC and SusD) are involved in binding and import of a substrate. Carbohydrate active families are labelled as follows: glycoside hydrolase (GH) and carbohydrate esterase (CE).

Richard Ndubuisi
M.A.Sc. Candidate

B.Sc., 2011, University of Nigeria Nsukka

Supervisor: Levente Diosady

Research Highlights

Best Presentation (15-minutes), BioZone Research Symposium, 2017.

Catalytic hydrothermal production of renewable diesel and potential integration with protein isolation

Traditionally, the production of edible oil is based on solvent extraction using hexane. However, the toxicity and flammability of the organic solvent prompts the search for a safer and more sustainable process. Accordingly, a green approach, the aqueous extraction process (AEP) has been proposed as an alternative. This technique is based on the principle of immiscibility of water with the oil. Based on this method, an aqueous solution is contacted with the pulverized oil seeds and the resulting mixture fractionated into oil, solids and aqueous phases via centrifugation. The solids can be used as animal feed whereas food-grade protein can be isolated from the aqueous phase. However, low oil recovery due to the formation of protein-stabilized emulsion with the aqueous phase and excessive energy requirement for drying are challenges to commercialization.

In an original work, we demonstrated that renewable hydrocarbons within the diesel boiling range can be directly produced from the resulting AEP emulsion without the need for water removal. Typically, the production of renewable diesel, also known as green diesel is based on the use of prohibitively expensive catalysts based on precious metals such as Pt in an organic medium. This approach generally results in better yields but requires disproportionate utilization of hydrogen to realize appreciable selectivity to desired alkanes. In our work, however, we used cheaper, lab-made catalysts in a hydrothermal medium and realized good yields and selectivity (both >70%) at 305°C and minimal initial hydrogen input. My work involved four main stages. The first was re-engineering the high-pressure reactors and adapting to the high temperature, high-pressure (HTHP) decarboxylation reaction. The pressure vessels were previously used for low temperature hydrogenation of oil (i.e., hardening). The second involved the synthesis of the process catalyst whose activity towards the direct conversion of the miscella into fuel was tested in the third stage. Finally, relevant analytical methods were developed for quantitation. By using a hydrothermal medium and working under conditions relevant for aqueous reforming of glycerol into hydrogen, the initial hydrogen pressure in the batch reactor was limited. We have shown that it is possible to form an integrated, sustainable process (AEP-Decarboxylation/decarbonylation, i.e., AEP-DCOx) wherein renewable diesel production is coupled with food-grade protein isolation. Protein has a higher value than fuel so the process could become commercially viable. Moreover, renewable diesel has better fuel properties and it is completely fungible with current hydrocarbon infrastructures and will likely become a major surrogate for biodiesel in fuel blends for compression ignition (CI) engines. Conclusively, the results shed light on the solution to the main issues associated with AEP and lay the groundwork for further exploring this green process integration towards commercialization.

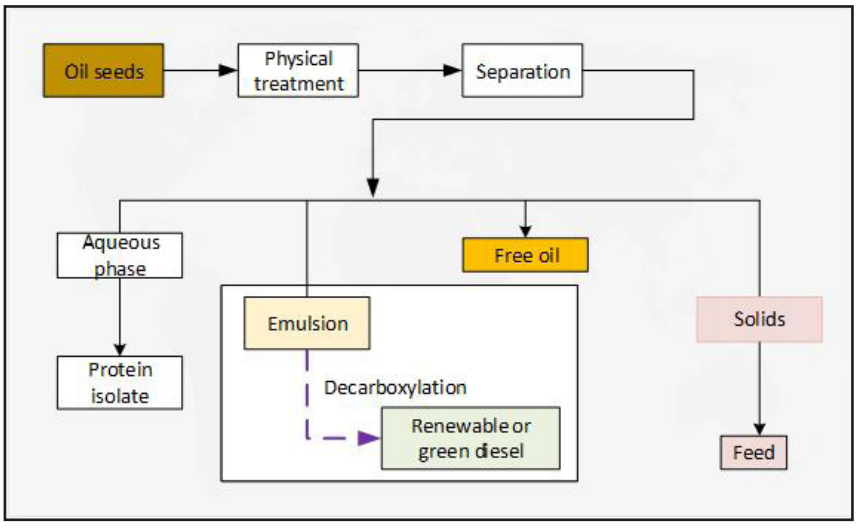


Figure 1: AEP-DCO$_x$ integrated process for simultaneous production of protein and diesel.

Research Highlights

P. H. Wang *et al.* (2016). Refined experimental annotation reveals conserved corrinoid autotrophy in chloroform-respiring *Dehalobacter* isolates. *ISME J.*, **11**, 626-640.

K. Nemr, R. Mahadevan. "Engineering a short, aldolase-based pathway for 1,3-butanediol production in *Escherichia coli*", poster presentation at the *Metabolic Engineering 11 Meeting*. Awaji, Japan. June 26-30, 2016.

K. Nemr, R. Mahadevan. "Modelling-based metabolic engineering strategy for biofuel and biochemical production in microbes", poster presentation at the *Advanced Biofuels Symposium (BioFuelNet)*. Ottawa, ON. May 27-29, 2014.

BioZone Graduate Scholarship, BioZone. (2014)

Kayla Nemr
Ph.D. Candidate

B.Sc. (Hons), 2012, University of Ottawa
B.A.Sc., 2012, University of Ottawa

Supervisor: Radhakrishnan Mahadevan

Designing aldolase-based biosynthetic pathways for biochemical production in *Escherichia coli*

Carbon-carbon bond formation is the cornerstone of many processes, specifically to synthesize longer, complex compounds from simple building blocks. Aldol reactions are important carbon-carbon bond condensation reactions in organic chemistry, but they suffer from the lack of specificity and stereocontrol. On the other hand, biochemical routes using aldolase-catalyzed reactions present an alternative to chemical synthesis by allowing for stereo-controlled synthesis under milder conditions. While many biosynthetic pathways have been reported in literature to produce various biofuels and biochemicals, some require long, complicated biosynthetic pathways. Optimizing strains that express such pathways can require cumbersome engineering strategies. However, aldolase-based pathways can offer an alternative shorter and simpler pathway design. Therefore, with the help of a pathway prediction algorithm, we designed new biosynthetic pathways employing aldolases as the key step. Ultimately, we plan to express the newly designed pathways in *Escherichia coli* for biochemical production directly from sugars or other renewable resources.

I plan to explore the potential of aldolase-based pathways for biochemical production by first demonstrating a short, simple pathway for 1,3-butanediol (1,3-BDO) production in *E. coli*. This requires enzyme characterization to identify candidates for the pathway, synthetic biology tools to construct and express the pathway in the host strain. By combining rational and model-based metabolic engineering strategies, the 1,3-BDO producing strain can be optimized for improved production.

To our knowledge, this is the first report of employing a short, two-enzymatic step aldolase-based pathway to synthesize 1,3-BDO directly from sugars. This strategy also offers insights into challenges that need to be overcome for using aldolase-based pathways that require aldehyde intermediates.

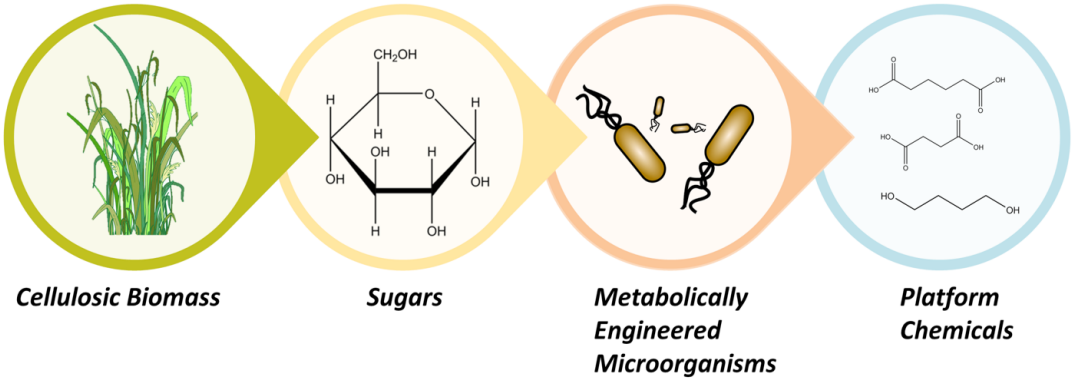
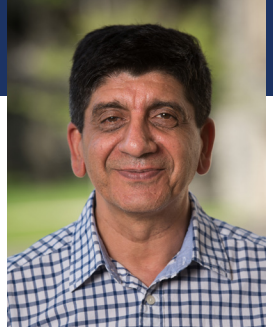


Figure 1: Basic pathway for biochemical production by microorganisms from renewable resources.



Mehdi Nouraei
Ph.D. Candidate

B.Sc., 1993, Isfahan University of Technology
M.Sc., 1996, Shiraz University
M.A.Sc., 2013, University of Toronto

Supervisor: Levente Diosady
Co-Supervisor: Edgar J. Acosta

Self-micro emulsifying delivery systems

Many newly-discovered drugs and nutraceuticals are hydrophobic in nature. When these drugs and nutraceuticals are ingested, their low aqueous solubility limits their absorption. To overcome this limitation, the active ingredient needs to be in a solubilized state in a delivery system. We have developed and evaluated a platform for a Self-Micro Emulsifying Delivery System (SMEDS) that upon exposure to intestinal fluids, form nano-sized droplets (microemulsion) loaded with active ingredient. The *in vivo* experiments showed that this system increases the bioavailability by three-fold. Furthermore, a solid version (powder) and semisolid version (gel) of this delivery system are developed with delayed release and extended release profiles. The platform is also efficient in extracting lipophilic components from plant material, including fruit peels and vegetables. This novel process can turn agri-food wastes/by-products into value-added products without using organic solvents.

Research Highlights

M. Nouraei, E. J. Acosta. (2017). Predicting solubilisation features of ternary phase diagrams of fully dilutable lecithin linker microemulsions. *J. Colloid Interface Sci.,* **495**, 178-190.

M. Nouraei. “Self-dispersing organogel”, oral presentation at the *90th ACS Colloid and Surface Science Symposium*. Cambridge, Massachusetts. June 5-8, 2016.

M. Nouraei, E. Acosta, L. L. Diosady. “Microemulsions as extracting and delivery systems for nutraceuticals”, oral presentation at the *OMAFRA Product Development Research Day*. Guelph, ON. March 7, 2016.

M. Nouraei, E. Acosta, L. Diosady. “HLD-NAC guided formulation of self-micro emulsifying delivery system (SMEDS)”, oral presentation at the *106th AOCS Annual Meeting & Expo*. Orlando, Florida. May 4-7, 2015.

Research Highlights

J. A. Obnamia, H. L. MacLean, B. A. Saville. "Comparison of LCA modelling tools for lignocellulosic biofuels: A case study on corn stover ethanol", poster presentation at the *BIO 12th Annual World Congress on Industrial Biotechnology*. Montreal, QC. July 19-22, 2015.

J. A. Obnamia, H. L. MacLean, B. A. Saville. "Comparative evaluation of LCA models on biofuels: A case study on corn stover ethanol", oral presentation at the *65th Canadian Chemical Engineering Conference*. Calgary, AB. October 4-7, 2015.

McLean Foundation Graduate Scholarship in Science and Technology, University of Toronto; Dept. of Chemical Engineering. (2016, 2017)

Eric David Baker Krause Graduate Fellowship, University of Toronto; School of the Environment. (2016)

Eco-Tec Founder's Scholarship, University of Toronto; Dept. of Chemical Engineering. (2016)

Jon Albert Obnamia
Ph.D. Candidate

B.A.Sc., 2011, University of Toronto
M.A.Sc., 2014, University of Toronto

Supervisor: Bradley Saville
Co-Supervisor: Heather MacLean

Environmental impacts of transportation biofuels

Global CO_2 emission rate from fossil fuel combustion has reached 32 billion tonnes per year, from which transportation contributes at least 22-24% to the total greenhouse gas (GHG) emissions. Within transportation GHG emissions, 75% typically comes from road transport while 11% comes from aviation transport. Reducing global GHG emissions from fuels can be achieved using less carbon intensive biofuels such as ethanol from lignocellulosic biomass and jet fuel from oilseeds, which can substitute or displace conventional fossil fuels. **To ensure that biofuels being used actually provide the GHG emission reduction benefit they promise, a comprehensive life cycle assessment of the biofuel is necessary to critically evaluate a biofuel's environmental impacts.**

Life Cycle Assessment (LCA) has become a widely accepted method for evaluating the environmental impacts of a product, and there have been many studies applying LCA to biofuel systems. **However, LCA studies on biofuels in the literature have shown that differences in LCA modelling data, choices, and assumptions often lead to drastic differences in environmental performance, even for the same biofuel product.** Some of the differences in data used in LCA modelling of biofuels have real-world validity (e.g., differences in product yield, carbon footprint of electricity grid used), but there are also LCA elements that contribute to differences in environmental impacts simply based on preferred LCA modelling choices that currently have no accepted consensus.

This research seeks to make a contribution in the LCA of biofuels by identifying and quantifying key sources of variation and uncertainty in LCA modelling of biofuels, whether based on feedstock choices, technology pathways, or LCA elements (e.g., model data, choices, and assumptions). The research also aims to utilize expertise gained from biofuel LCA studies to assess the environmental impacts of specific biofuel pathways that have yet to be explored (e.g., Canadian canola to biojet fuel pathways).

Overall, this research will serve to bridge overarching biofuel environmental objectives, as established by policies and regulations, with the necessary engineering activities related to the research, development, and deployment of biofuels. Ideally, this research will provide guidance to both engineering design and policy development.

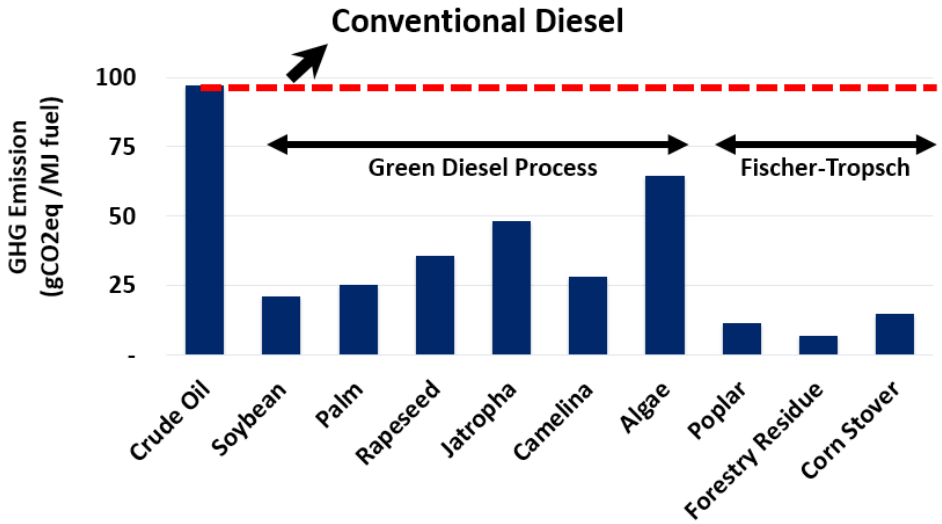


Figure 1: Survey of life cycle GHG emissions of petroleum diesel compared to renewable diesel from different biomass and technology pathways.

Kylie O'Donnell
Ph.D. Candidate

B.E.Sc., 2011, University of Western Ontario
M.A.Sc., 2014, University of Toronto

Supervisor: Emma Master

Breaking down barriers to hydrophobin-mediated surface functionalization

Hydrophobins (HFBs) are small (7-20 kDa), self-assembling, surface-active fungal proteins that assemble at hydrophobic-hydrophilic interfaces to form highly stable films. HFBs have been described as the most surface-active proteins known and represent non-immunogenic proteins with the capacity to change the property of surfaces via self-assembly as monolayers at liquid-solid interfaces. HFBs represent an attractive surface modification technology that can be paired with a variety of fusion partners to increase their versatility as surface modifiers. The utilization of HFBs is a recent trend in industrial applications such as coatings, various dispersion applications, stabilizing emulsions, personal care products, separation technologies, biosensors and electrodes, biomaterials, etc. We have looked at the effect of linker length and chemistry between a HFB and a functional tag in an attempt to improve upon the application of HFBs utilized in bio-sensing applications. While many attractive potential applications of HFBs exist, little is known about the impact of differentiating physical and environmental effects on their biochemical characterization. Additionally, there are significant differences in how individual HFBs function and many HFB characteristics are dependent on the experimental apparatus used. Therefore, we are also systematically characterizing a set of HFBs over various environmental parameters utilizing uniform characterization methods. This will provide the basis to have the ability to tune HFB assembly to surfaces based on chosen environmental parameters.

Research Highlights

J. A. Gandier *et al.* (2017). Characterization of a *Basidiomycota hydrophobin* reveals the structural basis for a high-similarity Class I subdivision. *Sci. Rep.,* **7**.

S. Javaherian, K. A. O'Donnell, A. P. McGuigan. (2011). A fast and accessible methodology for micro-patterning cells on standard culture substrates using Parafilm™ inserts. *PLoS One,* **6**.

K. O'Donnell, E. Master. "Construction of FcHyd5p variants to assess the potential of these class II hydrophobins as molecular tools for surface modification", poster presentation at the *Ontario-Quebec Biotechnology Meeting*. Toronto, ON. May 15-16, 2014.

NSERC Postgraduate Scholarships-Doctoral Program, NSERC. (2013-2016)

NSERC Canada Graduate Scholarships - Master's Program, NSERC. (2012)

Ontario Graduate Scholarship, Government of Ontario. (2011)

Research Highlights

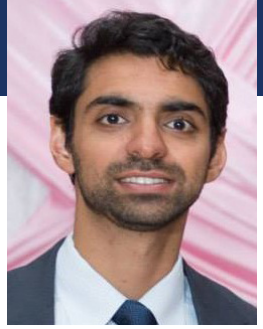
A. V. Pandit, S. Srinivasan, R. Mahadevan. (2017). Redesigning metabolism based on orthogonality principles. *Nat. Commun.,* **8**.

A. V. Pandit *et al.* "Complete biosynthesis of adipic acid in *S. cerevisiae*", poster presentation at the *International Conference on Biomolecular Engineering*. San Diego, California. January 8-11, 2017.

A. V. Pandit, R. Mahadevan. "Orthogonal Design of Metabolic Pathways", poster presentation at the *Metabolic Engineering Conference – XI*. Kobe, Japan. June 26-30, 2016.

A. V. Pandit, S. Srinivasan, R. Mahadevan. "Orthogonal design of metabolic pathways" in Metabolic Engineering *11. (2016)*, pp. 474-475.

A. V. Pandit, R. Mahadevan. (2011). *In silico* characterization of microbial electrosynthesis for metabolic engineering of biochemicals. *Microb. Cell Fact.,* **10**.



Aditya Vik Pandit
Ph.D. Candidate

B.A.Sc., 2009, University of Toronto
M.A.Sc., 2011, University of Toronto

Supervisor: Radhakrishnan Mahadevan

Engineering carbon fixation metabolic pathways

Amidst the many mechanisms present in nature, two routes present themselves from the outset as being the most viable for heterologous demonstration of microbial electrosynthesis in *E. coli*. The first is the assimilation of electrical energy by external mediators including neutral red to aid the direct assimilation of CO_2 by metabolic pathways. The second is the assimilation of reduced carbon species, which are derived from electrochemical sources, by metabolic pathways. These two routes are the primary focus of my research.

Successful demonstration would allow bioprocesses to become efficient at producing chemicals and fuels directly from CO_2. This would address some of the economic challenges facing current bioprocesses that are struggling with high feedstock costs and low cost of oil which makes them uncompetitive.

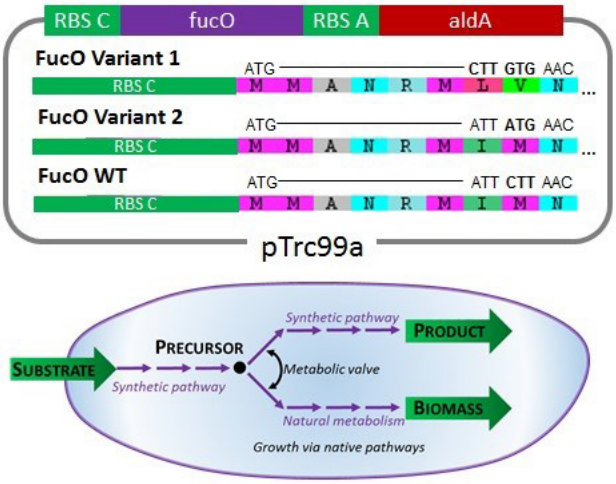
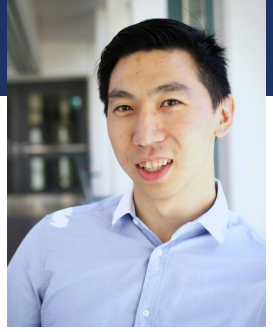


Figure 1: Research overview.



James Poon
Ph.D. Candidate

H.B.Sc., 2010, University of Toronto
M.Sc., 2012, University of Guelph

Supervisor: Alison McGuigan
Co-Supervisor: Thomas Waddell

Manipulating micro-physical cues for tissue engineering

There are currently no acceptable treatments for injuries affecting long segments of the trachea. Tissue-engineered scaffolds are a promising alternative but have not yet been optimized to incorporate functional epithelium. Airway epithelium contains multiciliated cells that coordinate their beating to eliminate mucus and foreign particles. Currently, air-liquid-interface (ALI) culture is used to generate artificial adult airway epithelium *in vitro*. This culture system provides the apical-basal polarization necessary for differentiation of progenitors into their terminal cell types. However, ALI culture does not produce planar polarized epithelium with correct alignment and beating of motile cilia.

We are particularly interested in using the physical microenvironment to direct cell function. Airway epithelium rests on a basement membrane of aligned collagen fibres that provide topographical cues at the scale of the individual cells. Using elastomeric and hydrogel substrates, we are assessing the ability of primary human tracheal epithelial cells to polarize and differentiate into mature epithelial cells, under ALI, on defined microgrooved substrates of varying pitch and depth. Super-resolution microscopy combined with computational analysis techniques are utilized to study cilia orientation. Our goal is to produce epithelial tissue with concerted cilia function using substrates with optimized grooved features.

Understanding topographical requirements will improve clinical impact, by providing the basis for generating gel coatings with specific properties for lining the lumens of engineered airway replacements to guide epithelial organization.

Research Highlights

J. C. H. Poon *et al.* "Biomimetic surfaces for preservation of alveolar type II cells", poster presentation at the *Medicine by Design Symposium*. Toronto, ON. November 28, 2016.

J. C. H. Poon *et al.* "Controlling the microenvironment of human airway epithelial cells for tracheal tissue engineering", poster presentation at the *10th World Biomaterials Congress*. Montreal, QC. May 17-22, 2016.

A. C. Paz *et al.* (2014). Challenges and opportunities for tissue-engineering polarized epithelium. *Tissue Eng. Part B. Rev.,* **20**, 56-72.

NSERC Postgraduate Scholarships-Doctoral Program, NSERC. (2013-2016)

Wildcat Graduate Scholarship, University of Toronto; Institute of Biomaterials & Biomedical Engineering. (2012)

NSERC Canada Graduate Scholarships - Master's Program, NSERC. (2017)

Scott Proulx
M.A.Sc. Candidate

B.A.Sc., 2016, University of Ottawa
B.Sc., 2016, University of Ottawa

Supervisor: Radhakrishnan Mahadevan

Adipic acid production by *E. coli*

Production of high-value chemicals has been rooted in the petrochemical industry for the entirety of the 20th century until present day. As fossil fuel reserves begin to decline and as a result of the pressing issue of climate change, alternative processes are desired. Such a process should produce the desired chemical using sustainable, non-toxic materials without compromising economic efficiency. Adipic acid, which is a precursor for nylon production, presents such a challenge.

The use of microbial organisms such as yeast and bacteria as biocatalysts to produce chemicals is a solution that would use sustainable feedstocks such as glucose, and may be operated at moderate reaction conditions with minimal toxic byproducts. However, organisms will not normally produce these chemicals in high enough quantities on their own. By engineering the metabolism of a microorganism, the enzymes that are expressed may be configured to optimally produce a product. The chemical that my research will focus on is adipic acid produced in *Escherichia coli*.

It is clear that an ideal strain of *E. coli* would produce adipic acid at high yield, titer and productivity. Although initial research may begin using glucose as a carbon source, the eventual use of alternative feedstocks such as ethylene glycol is desired.

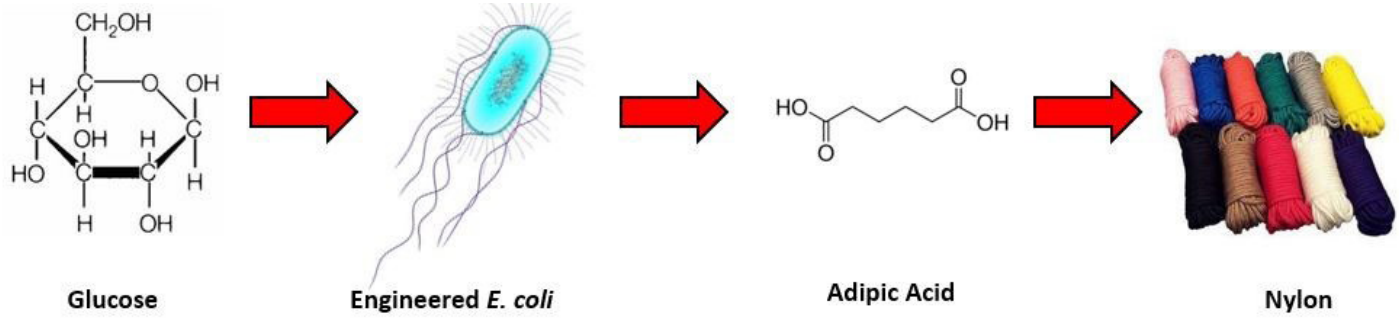


Figure 1: Adipic production pathway using E. coli

Luz Adriana Puentes Jacome
Ph.D. Candidate

B.Eng., 2008, Universidad Pontificia Bolivariana
M.A.Sc., 2012, Carleton University

Supervisor: Elizabeth Edwards

Anaerobic biodegradation of chlorobenzenes and lindane

Chlorinated organic compounds such as chlorobenzenes and lindane, the γ isomer of hexachlorocyclohexane (γ-HCH), are environmentally-regulated persistent organic pollutants. Chlorobenzenes are used as intermediates for the synthesis of various chemicals and pesticides. They are found in the environment as a result of uncontrolled industrial discharges and the transformation of other chlorobenzenes or pesticides. In fact, lindane, historically used as an insecticide in agricultural crops, can be transformed and partially dechlorinated by microorganisms into a mixture of different chlorobenzenes. Lindane has been classified by the International Agency for Cancer Research as carcinogenic. Chlorobenzenes and lindane are somewhat hydrophobic. They are found as soil, sediment, and groundwater contaminants in anaerobic environments where they may be reductively dechlorinated by bacteria. Currently, little is known about the physiology of the bacteria and the associated enzymes (reductive dehalogenases or RDases) carrying out this process. Also, engineers need to develop field site remediation strategies that will accommodate their hydrophobic nature and limited bioavailability. This project is providing insight into these engineering and scientific questions by studying three dechlorinating microbial enrichment cultures: KB-1, MCB/Benzene, and GT1. KB-1, commercially used at contaminated sites, transforms aliphatic chlorinated compounds into non-toxic ethene gas. The MCB/Benzene culture biodegrades monochlorobenzene (MCB) and benzene into methane and carbon dioxide. The GT1 culture degrades lindane to MCB and benzene. We evaluate the dechlorination potential of these mixed cultures against chlorobenzenes and lindane while monitoring growth and changes in the microbial populations. Our specific goals are to: (i) study the reductive dechlorination of chlorobenzenes by the KB-1 culture; (ii) investigate the bioconversion of lindane by a mixed consortium of GT1 and MCB/Benzene-degrading organisms; (iii) identify unknown RDase(s); and (iv) explore the use of activated carbon to deliver a lindane-degrading consortium to lindane-contaminated sediment.

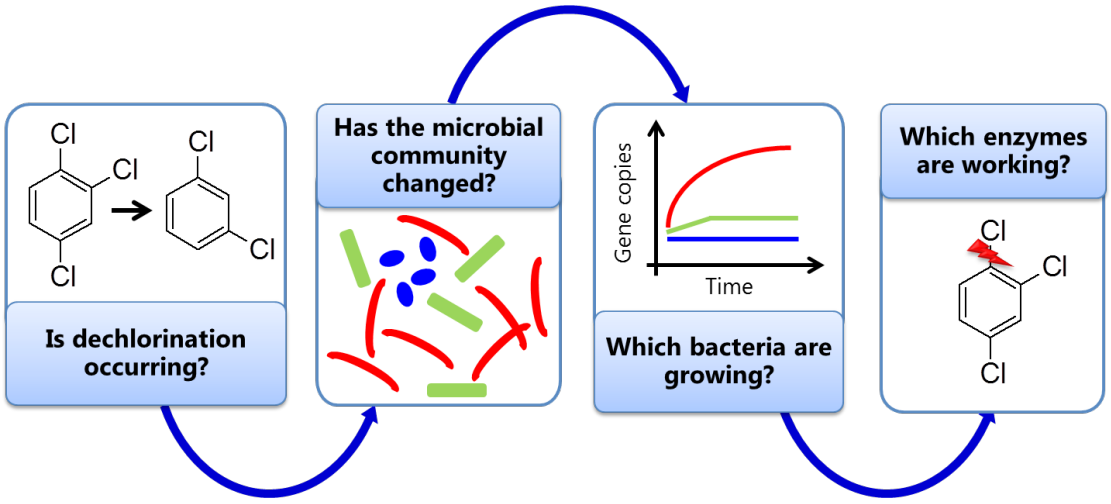


Figure 1: Flow process of studying microbial remediation cultures that dechlorinate pollutants.

Research Highlights

L. A. Puentes Jacome, P. Wang, L. Lomheim, E. A. Edwards. "Microbial community changes in response to 1,2,4-trichlorobenzene dechlorination based on 16S amplicon pyrosequencing of DNA and cDNA in KB-1, a mixed microbial culture used for bioremediation", poster presentation at the *International Society for Microbial Ecology Conference*. Montreal, QC. August 21-26, 2016.

L. A. Puentes Jacome *et al.* "Biodegradación anaerobia de clorobenzenos y pesticidas organoclorados: aplicación de técnicas de química analítica y biología molecular", oral presentation at the *Seminario de Ingeniería Ambiental, Universidad Ponitificia Bolivariana*. Bucaramanga, Colombia. October 12-14, 2016.

Ontario Graduate Scholarship, Government of Ontario. (2014-2017)

BioZone Graduate Scholarship, BioZone. (2013-2014)

Research Highlights

A. T. Quaile *et al.* "Overcoming the challenges of activating secondary metabolite gene clusters in *Aspergillus niger*", oral presentation at the *Industrial Biocatalysis Network: Annual General Meeting*. Montreal, QC. May 18-19, 2016.

M. L. Urbanus *et al.* (2016). Diverse mechanisms of metaeffector activity in an intracellular bacterial pathogen, *Legionella pneumophila*. *Mol. Syst. Biol.,* **12**.

O. Molenda, A. T. Quaile, E. A. Edwards. (2016). *Dehalogenimonas* sp. Strain WBC-2 genome and identification of its trans-dichloroethene reductive dehalogenase, TdrA. *Appl. Environ. Microbiol.,* **82**, 40-50.

A. T. Quaile *et al.* (2015). Molecular Characterization of LubX: Functional Divergence of the U-Box Fold by *Legionella pneumophila*. *Structure,* **23**, 1459-1469.

Andrew Quaile
Postdoctoral Fellow

B.Sc. (Hons), 2005, University of Liverpool
Ph.D., 2009, University of Liverpool

Supervisor: Alexei Savchenko

Understanding pathogenesis through functional characterization of bacterial effectors

The bacterial effectors are a group of proteins that are translocated by specialized bacterial machinery into their hosts. They are critical determinants of virulence thanks to their ability to induce, repress, hijack and subvert the cellular responses of the host to the invader. Discovering and understanding the *modus operandi* of these proteins is often obfuscated by their low sequence similarity with functionally characterized proteins. Consequently, the structural data can provide the first insight into what these proteins do. However further understanding of their function requires identification of specific host proteins and processes targeted by these pathogenic factors.

To answer this challenge, I am taking advantage of the high quality, highly-purified protein we are able to produce and use them as bait in search for their host cell interactors. Using recently developed highly specific affinity tags and entirely gel-free complex purification methodology coupled with tandem mass spectrometry, I am able to identify not only the directly interacting partners, but also entire functional complexes without being limited by the availability of specific antibodies. Once I identify and confirm interactions between bacterial pathogenic factors and host proteins, we move into characterization of the cell processes and functions affected by bacterial infection. This information is critical to our general understanding of bacterial pathogenesis as well for development of novel antimicrobial therapies.

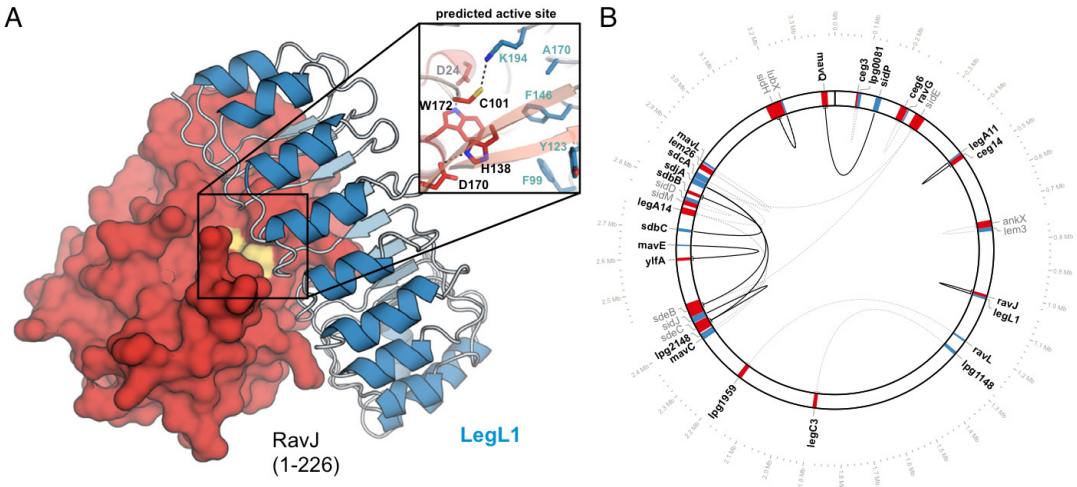


Figure 1: Physical and genetic interaction studies reveal protein-protein interactions in Legionella pneumophila. A) Effector LegL1 (blue) acts as a direct antagonist of RavJ (red) by blocking its active site (PDF:4XA9). B) Diagram of effector-effector suppression pairs.

Darren Rodenhizer
Ph.D. Candidate

B.A.Sc., 2012, University of Toronto

Supervisor: Alison McGuigan

Tissue-engineered tumour models

The tumour microenvironment is heterogeneous and consists of multiple cell types, variable extracellular matrix (ECM) composition, and contains cell-defined gradients of small molecules, oxygen, nutrients and waste. Emerging *in vitro* cell culture systems that attempt to replicate these features often fail to incorporate design strategies to facilitate efficient data collection and stratification. By combing cutting-edge tissue engineering principles and device design ingenuity, my work strives to create smart-data-acquisition tissues for use in drug screening platforms. Our first solution - the tissue roll for analysis of cellular environment and response (TRACER) - is a 3D tissue that can be rapidly taken apart for analysis. TRACER enabled our team to spatially map cell metabolism in concert with cell phenotype in a 3D tissue; for the first time. We envision this technology will provide a platform to create complex, yet controlled tumour microenvironments that can be easily disassembled for snapshot analysis of cell phenotype and response to therapy in relation to microenvironment properties.

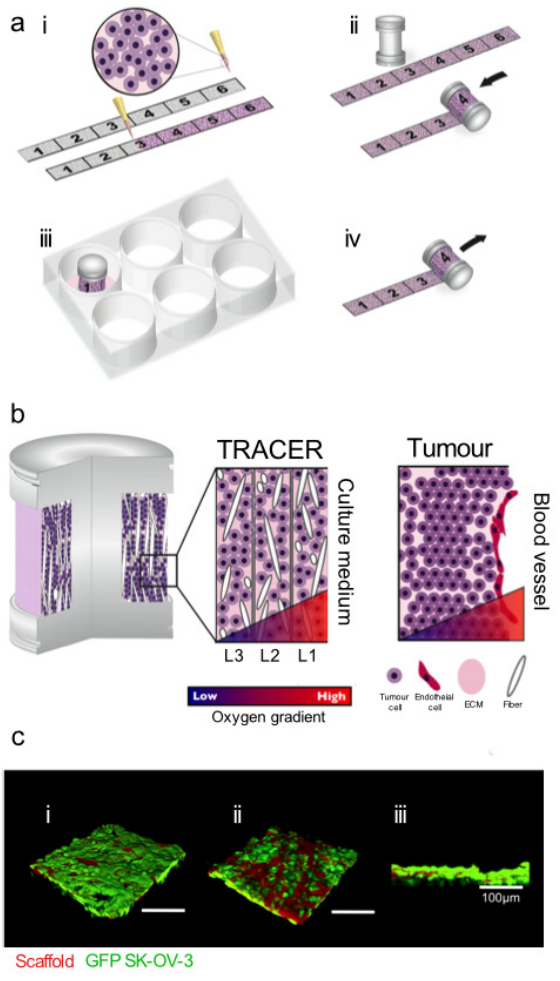


Figure 1: Tracer Concept. ***ai - aiv)*** *Assembly of TRACER by infiltration of cells into a paper scaffolding strip followed by rolling to assemble and unrolling to disassemble the model.* ***b)*** *Oxygen gradients are established in TRACER through metabolic consumption by the cells in a layer-wise manner.* ***c)*** *Cells (green) infiltrate void spaces in the paper scaffold network (fibres shown in red) to generate dense tissue mimetic layers.*

Research Highlights

D. Rodenhizer *et al.* (2016). A three-dimensional engineered tumour for spatial snapshot analysis of cell metabolism and phenotype in hypoxic gradients. *Nat. Mater.,* **15**, 227-234.

D. Rodenhizer, D. Cojocari, B. G. Wouters, A. P. McGuigan. (2016). Development of TRACER: Tissue roll for analysis of cellular environment and response. *Biofabrication,* **8**.

D. Rodenhizer *et al.* "TRACER: A 3D engineered tumour for quantifying spatial metabolic reprogramming in hypoxic gradients", oral presentation at the *American Society for Mass Spectrometry Meeting*. San Antonio, Texas. June 5-9, 2016.

NSERC Postgraduate Scholarships-Doctoral Program, NSERC. (2016)

Frank Howard Guest Bursary, University of Toronto; Dept. of Chemical Engineering. (2016)

Research Highlights

Ontario Graduate Scholarship, Government of Ontario. (2016-2017)

Ontario Graduate Scholarship, Government of Ontario. (2012-2013)

NSERC Canada Graduate Scholarships - Master's Program, NSERC. (2011-2012)

NSERC Undergraduate Student Research Awards, NSERC. (2010)

NSERC Undergraduate Student Research Awards, NSERC. (2008)

Fawzi Salama
Ph.D. Candidate

B.A.Sc., 2011, University of Ottawa
M.A.Sc., 2013, University of Ottawa

Supervisor: Radhakrishnan Mahadevan

Dynamic metabolic modeling of microbial communities

Microbial communities, assemblies of more than one interacting microbial species, are essential to human and environmental heath. Communities in the soil provide nutrients to plants, communities in the ocean help regulate the planet's carbon balance, and communities in the human body aid in the digestion of fibers and provide essential short-chain fatty acids. Typically, however, microbial communities are composed of hundreds or thousands of members. While individual species can be isolated and studied *in vitro*, communities composed of a large number of species are difficult to study using currently available experimental techniques. Mathematical modeling can aid in this task.

Using the sequenced genome and other biochemical data, the network of metabolic reactions in a species can be constructed, and flux balance analysis (FBA) can be used to construct predictive models for a single species [1]. Furthermore, Dynamic FBA (dFBA), an extension of FBA, can be used to account for dynamic behaviours [2]. The goal of my research is to apply this technique and others to the modeling of microbial communities by improving, extending and generalizing previous efforts in this regard [3]. This research will aid in the study of natural microbial communities. It will also aid in the exploitation of synthetic communities for industrial applications, in which there has been growing interest in recent years.

[1] Orth, J. D., Thiele, I. and Palsson, B. (2010). What is flux balance analysis? *Nature Biotechnology,* 28:245-248.

[2] Mahadevan, R., Edwards, J. S. and Doyle, F. (2002). Dynamic flux balance analysis of diauxic growth in *Escherichia coli. Biophysical Journal*, 83:1331-1340.

[3] Zhuang, K. et al. (2011). Genome-scale dynamic modeling of the competition between *Rhodoferax* and *Geobacter* in anoxic subsurface environments. *ISME Journal*, 5:305-16.

Juveria Siddiqui
Postdoctoral Fellow

M.Sc., 1997, University of Karachi
M.Phil., 2006, University of Karachi
Ph.D., 2011, University of Karachi

Supervisor: Levente Diosady

Micronutrients fortification through development and optimization of iron-containing reverse-enteric coated micro-particles using spray drying technique

Iron deficiency anemia is the most common and most widespread nutritional disorder, affecting more than two billion people worldwide. While anemia has a global presence, it is much more prevalent in developing countries. Insufficient intake of iron-rich animal food, and a diet deficient in other proteins and ascorbic acid, which helps in non-heme iron absorption, are responsible for 50% of all causes of anemia. The consequences of iron deficiency in children are stunted growth and slowed mental development, as well as increased risk of infections due to immune system dysfunction. In developing countries, anemia in women of child bearing age is reported to result in increased mortality in childbirth. Furthermore, iron deficiency leads to decreased absorption of iodine and vitamin A, which causes major additional nutritional disorders.

Iron deficiency is preventable. It is addressed through three main approaches: fortification, supplementation, and dietary interventions. Among them, food fortification is the least expensive, while providing an effective method for increasing the iron intake in diet without compromising dietary patterns. A number of foods have been fortified with iron and the successful reduction of mortality was observed in populations that adopted this strategy. It is accepted as cost-effective and even more importantly, it is available to all because staple food is fortified.

Food fortification is a highly recommended and cost-effective method for addressing nutrient deficiency all over the world. Finding the appropriate carrier to fortify is the focus of recent research in food product development. The fundamental challenge is to fortify food with nutrients so that they can be delivered, are bio-available and do not react with other food components present, interactions can induce loss of flavour, unpleasant colour and taste or reduce the nutritional value of food. Microencapsulation, using spray drying technique, is the method of choice as nutrients can be packaged into a coating material and content released into the stomach or intestine. In my present research focus, tea is proposed as potential vehicle for micronutrient fortification joining other staple foods like sugar, salt, rice and wheat. Tea is a widely consumed and well accepted beverage. However, using it as a fortification vehicle is technically challenging due to the presence of polyphenolic compounds, which can bind nutrients thus possibly reducing its bio-availability and changing the taste and flavor.

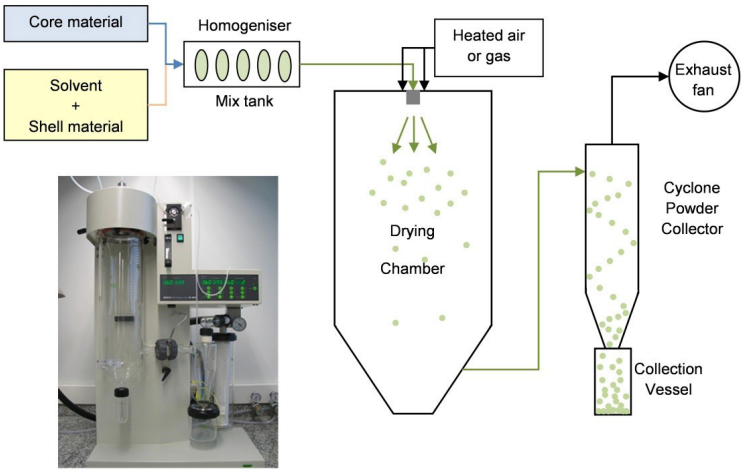


Figure 1: Microencapsulation using spray drying method.

Research Highlights

P. J. Stogios *et al.* (2016). Rifampin phosphotransferase is an unusual antibiotic resistance kinase. *Nat. Commun.,* **7**, 1-12.

M. Rolando *et al.* (2016). *Legionella pneumophila* S1P-lyase targets host sphingolipid metabolism and restrains autophagy. *Proc. Natl. Acad. Sci. U.S.A.,* **113**, 1901-1906.

R. L. Sontag *et al.* (2015). Electroporation of functional bacterial effectors into mammalian cells. *J. Visualized Exp.*

A. T. Quaile *et al.* (2015). Molecular Characterization of LubX: Functional Divergence of the U-Box Fold by *Legionella pneumophila*. *Structure,* **23**, 1459-1469.

E. S. Nakayasu *et al.* (2015). Identification of *Salmonella* typhimurium deubiquitinase SseL substrates by immunoaffinity enrichment and quantitative proteomic analysis. *J. Proteome Res.,* **14**, 4029-4038.

Tatiana Skarina
Laboratory Technician

M.Sc., 1983, Novosibirsk State University

Supervisor: Alexei Savchenko

Protein production and crystallization

I work to produce single protein and protein complex crystals suitable for 3D structure determination by x-ray crystallography, the main experimental tool to obtain atomic resolution details of biomolecules that contributes over 90% of all structures in the protein data bank.

A single crystal structure of a protein is not always enough to completely understand the molecular function because almost all proteins interact with small molecules or other proteins to carry out their function. The importance of protein interaction reflects the importance of crystallization of protein-ligand and protein-protein complexes.

My work is complementary and foundational for additional experimental techniques because 3D atomic details of biomolecules provide powerful information for discovery and understanding of their function, informs engineering to manipulate their properties, and informs drug discovery efforts to inhibit their activity.

Protein complexes crystallization requires optimization of multiple parameters (buffer, pH, salt and precipitant conditions, selection and ratio of ligand to protein, incubation time and temperature, type of crystallization setup) to increase crystal quality and suitability for the x-ray diffraction experiment.

My major research advances have been to play a central role in structural genomics initiatives by producing structure determination quality crystals for over 300 different proteins. Among them are proteins involved in the infectious process by human pathogens such as *Legionella pneumophila*, enzymes dispersed in antibiotic-resistant bacteria that covalently modify and inactivate antibiotics (including rifamycins, aminoglycosides, macrolides), CRISPR-Cas system proteins, and many enzymes involved in biocatalysis. I work on projects in the NSERC Industrial Biocatalysis Network (IBN), Center for Structural Genomics of Infectious Diseases (CSGID) and the Ontario Research Fund (ORF) project "Solving the Antibiotic Resistance Crisis".

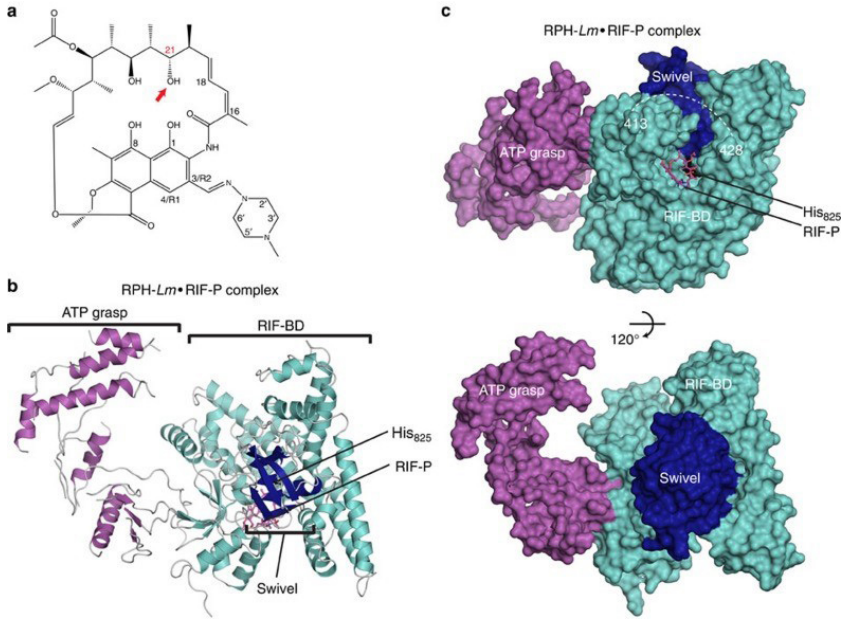
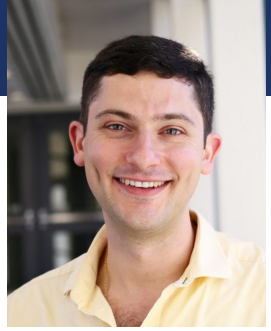


Figure 1: Structure of the RPH-Lm·RIF-P complex. (a) Chemical structure of RIF; the site of phosphorylation by RPH proteins is shown in red and indicated with an arrow. (b) Structure of the RPH-Lm·RIF-P complex. ATP-grasp (residues 1–314), RIF-binding (RIF-BD; residues 327–754) and swivel phosphohistidine (residues 767–865) domains are shown in shades of purple, cyan and dark blue, respectively. RIF-P is shown in pink sticks. (c) Surface representation of the RPH-Lm·RIF-P complex, the bottom view is rotated ~120° from the top view. Dashed lines indicate a large disordered region between residues 413 and 428 in the RIF-BD. RIF-P is shown in pink sticks and the catalytic histidine labelled (His825).



John Soleas
Ph.D. Candidate

B.M.Sc., 2010, University of Western Ontario
M.Sc., 2012, University of Toronto

Supervisor: Alison McGuigan
Co-Supervisor: Thomas Waddell

Architecture can manipulate the differentiation of lung progenitor cells

Mechanical forces are essential for normal lung development. During epithelial differentiation *in vivo*, the proximal (SOX2+), pseudostratified airway epithelium develops in tube diameters larger than those found in the distal (SOX9+), squamous epithelium. We hypothesized that lung progenitor cells grown in cylinders smaller than 100µm will guide cells towards a distal fate. Our aim was to expose lung progenitors, derived from embryonic stem cells, to a defined architecture, in the form of hollow, three-dimensional cylinders of differing diameters to create cell-lined cavities reminiscent of the developing lung tubule and to study how this geometric configuration affects cell fate choice. Using photolithographic and moulding technologies, we have created hollow cylinders of 40-400µm in diameter with a height of 180µm in either 15% gelatin or polydimethylsiloxane. NKX2.1+FOXA2+SOX2+SOX9+ lung progenitors successfully form hollow cavities following 8 days of culture in both materials. Our data suggests that at a 100µm diameter distal fate choice SOX9+ (84.65±6.99%) is favoured over proximal SOX2+ (8.14±7.60%) or dual positive SOX2+SOX9 (11.58±7.14%) in gelatin hydrogels. Similar trends were seen in 80µm and 40µm diameter tubes. In flat control conditions, only 2.15±2.49% SOX9+, 1.00±1.11% SOX2+ were seen and 95.00±5.07% of cells remained dual positive. To assess whether local or paracrine signaling was affecting fate choice, we grew our cells on positive tubular architecture (posts) and found a similar affect. This observation suggests that curvature itself is guiding fate choice. Utilizing a tissue engineering approach to apply architectural cues to heterogeneous lung progenitors, we have guided cell fate choice.

Research Highlights

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S. Srinivasan, W. R. Cluett, R. Mahadevan. (2015). Constructing kinetic models of metabolism at genome-scales: A review. *Biotechnol. J.,* **10**, 1345-1359.

B.Tech., 2010, National Institute of Technology, Rourkela
M.Sc., 2012, University of Saskatchewan

Shyam Srinivasan
Ph.D. Candidate

Supervisor: Radhakrishnan Mahadevan
Co-Supervisor: William Cluett

Assessing the impact of bistability in metabolic networks for metabolic engineering design

Metabolic engineering can be used to design and build microbes that can be used as cell factories for sustainable bio-based production of various chemicals. However, living cells are complex systems that possess dynamic properties by virtue of the numerous interactions that occur within the cell and govern their behaviour. Metabolism is one of the interactions.

In model-based design for metabolic engineering, we used mathematical models of metabolism to study, predict and design microbial cell factories. However, metabolism can exhibit dynamic phenomena like-bistability due to the presence of regulatory motifs like the positive feedback loop. The presence of these motifs govern various aspects of cellular metabolism and enable microbial cells to be robust to various disturbances in their environments. However, current models lack the ability to account for these regulatory interactions, and are unreliable for the purpose of designing and predicting cellular responses for metabolic engineering.

Among the various dynamic properties of metabolism, my focus is on bistability, which is widely seen in biological networks. For instance, bistability is seen in the mitogen activated protein kinase signaling pathway, in mitochondrial respiration through the electron transport chain, as well as in the regulation of the lac operon in *Escherichia coli*.

Although bistability is known to confer an evolutionary advantage to microbes, the impact of bistability on the production characteristics of engineered strains used as cell factories has not been widely studied. Due to the complex nature of metabolic and gene regulatory networks, perturbations executed as part of realizing a cell factory design can have an adverse impact on the bistable production characteristics of the cell. These perturbations can be made either to enzyme expression or to enzyme regulation. However, so far no analyses have been done on the impact of these perturbations on the bistable production characteristics of the metabolic network.

To fill the aforementioned knowledge gaps, my goal is to develop model-based design strategies to improve production using bistable metabolic networks. To facilitate my study of bistability in metabolism, I also develop computational methodologies to improve existing kinetic models of metabolism by proposing superior parameter estimation techniques.

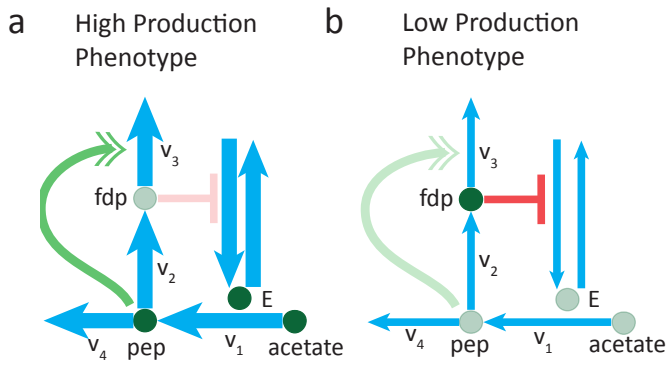

Figure 1: Research overview.

Peter Stogios
Research Associate

H.B.Sc., 2001, University of Toronto
Ph.D., 2010, University of Toronto

Supervisor: Alexei Savchenko

Molecular mechanisms of antibiotic resistance in pathogenic bacteria

Problem: Antibiotic resistance in pathogens threatens modern medicine. Understanding the molecular basis for resistance will allow for an understanding of how it arises, what genes to monitor, and how to inhibit these resistance-conferring enzymes. We also study the molecular basis for action of novel antibacterials.

Knowledge gaps: Many antibiotic resistance genes lack 3D structural information that is necessary to inform rational drug design. As well, new antibiotic resistance enzymes are regularly being discovered.

Solutions: 3D structural studies (x-ray protein crystallography) will allow for molecular images of these enzymes and provide insights into drug discovery. Enzyme activity assays will reveal substrate specificity and key active site features allowing for catalysis.

Commercialization: inhibitors of resistance enzymes or novel antibacterials that we study can become drug leads that with further optimization through medicinal chemistry, could act as combination therapy to treat antibiotic resistance.

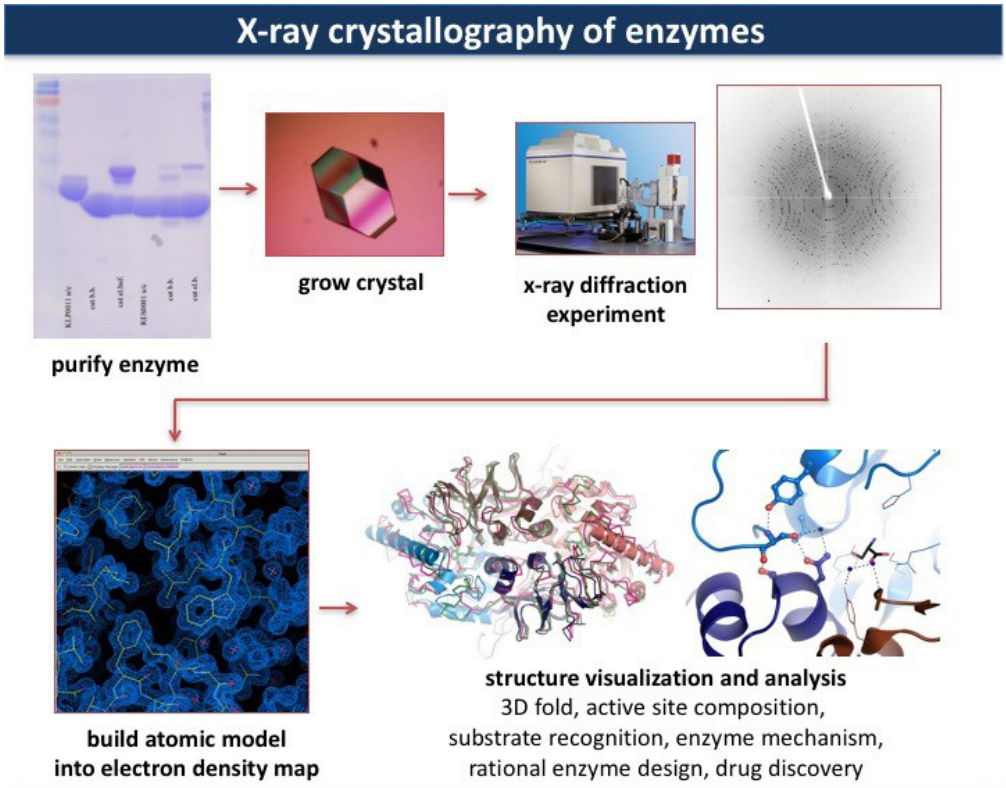

Figure 1: This diagram summarizes the main steps in how to solve the 3D structure of an enzyme using the technique of x-ray crystallography, which is the main technique employed in my research. Enzymes are purified from E. coli; crystals are grown using in-house suite of solutions; x-ray diffraction experiment is conducted at our x-ray lab or remotely at synchrotron sources; the data reveals a "map" showing the probability of observing atoms in 3D space; into this map is built the amino acid sequence of the enzyme. Finally, the 3D structure is analyzed for such features as active site composition and enzymatic mechanism. This information informs further experiments (not shown here) including rational enzyme design and drug discovery.

Research Highlights

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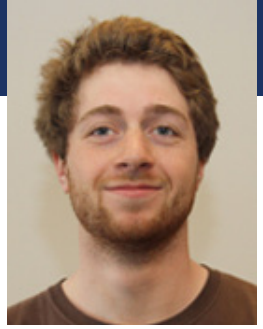

Dylan Valleau
Ph.D. Candidate

Supervisor: Alexei Savchenko

Characterization of molecular determinants of bacteria-host interactions

Dylan's research aims to elucidate how gram-negative bacterial pathogens colonize their host through injection of effector proteins into host cells. Under the supervision of Alexei Savchenko, his task is to characterize the role of ubiquitin protein ligase (E3 enzymes) effectors from pathogenic *E. coli*, *Salmonella*, and *Shigella*, through co-immunoprecipitation and mass spectrometry, structural characterization, and *in vivo* expression analysis in human cell lines.

B.Sc., 2000, National Agrarian University
D.E.A., 2003, University of Liège
Ph.D., 2009, University of Liège

Caroline Vanderghem
Postdoctoral Fellow

Supervisor: Bradley Saville
Co-Supervisor: Heather MacLean

Financial analysis and life cycle assessment of biofuels

Increased globalization and international trade has led to a steady rise in air travel and forecast demand for jet fuel. The International Air Transport Association commitment to reduce carbon emissions by 50% by 2050 compared to the 2005 level and the inclusion of aviation in the European Union Emissions Trading Scheme has created interest in biomass-derived jet fuel. Biomass-derived jet fuel from feedstocks grown in an economically sustainable manner with a minimum of arable land, holds long-term promise to help reduce aviation greenhouse gases (GHG) emissions and dependence on fossil fuels.

Among different pathways to produce biomass-derived jet fuel, the hydroprocessing pathway involves treatment of fats and oils, in the presence of hydrogen, and converted into hydroprocessed renewable jet (HRJ) fuel. The ASTM has approved HRJ as a drop-in fuel. The HRJ cost of production data and uncertainties analysis related to price volatility of fuel and renewable feedstocks remains scarce.

Our first goal is to evaluate the financial viability of HRJ production, from two low-input oilseed crops *Camelina sativa* (camelina) and *Brassica carinata* (carinata) and used cooking oil (UCO). A Monte Carlo analysis is performed to measure the robustness of the financial performance by taking into consideration key uncertainty parameters like capital cost, price of feedstock, and output fuel prices.

The second goal of this work is to determine to what degree HRJ can reduce GHG emissions to meet the industry's GHG target by 2050, in terms of the potential supply within the Canadian and global contexts. A life cycle assessment is performed comparing HRJ derived from camelina, carinata, and UCO and paired with a bottom-up supply assessment.

Research Highlights

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N. Venayak, N. Anesiadis, W. R. Cluett, R. Mahadevan. (2015). Engineering metabolism through dynamic control. *Curr. Opin. Biotechnol.*, **34**, 142-152.

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NSERC Postgraduate Scholarships-Doctoral Program, NSERC. (2014-2017)

NSERC CREATE in Manufacturing, Materials and Mimetics (M3), NSERC. (2014-2015)

Ontario Graduate Scholarship, Government of Ontario. (2012-2013)

Naveen Venayak
Ph.D. Candidate

B.A.Sc., 2012, University of Ottawa
B.Sc., 2012, University of Ottawa

Supervisor: Radhakrishnan Mahadevan

Applied laboratory automation and synthetic biology to engineer dynamically controlled microorganisms

Microorganisms show promise in solving many societal concerns in areas such as health, sustainability, energy and consumer products. One area which has received particular attention over the past 30 years is the use of microbes to produce high-value chemicals and fuels. Microbial metabolism provides a diverse network of chemicals, many of which have commercial applications, and many others which can be converted to useful compounds with additional biochemical conversions. However, the complexity of metabolism brings challenges in developing organisms for robust, scalable and economical bioprocesses. To overcome some of these limitations, significant efforts have been made to develop metabolic models for these organisms and to improve the throughput of experiments and data analysis. My work focuses on developing synthetic circuits to dynamically control microbes throughout the duration of a fermentation process, allowing finer control and significantly improved performance. In parallel, we have developed automated liquid handling methods and data analysis pipelines to expedite this process. This work has broad applicability to metabolically engineered organisms, as most are not dynamically controlled and suffer poor performance as a result.

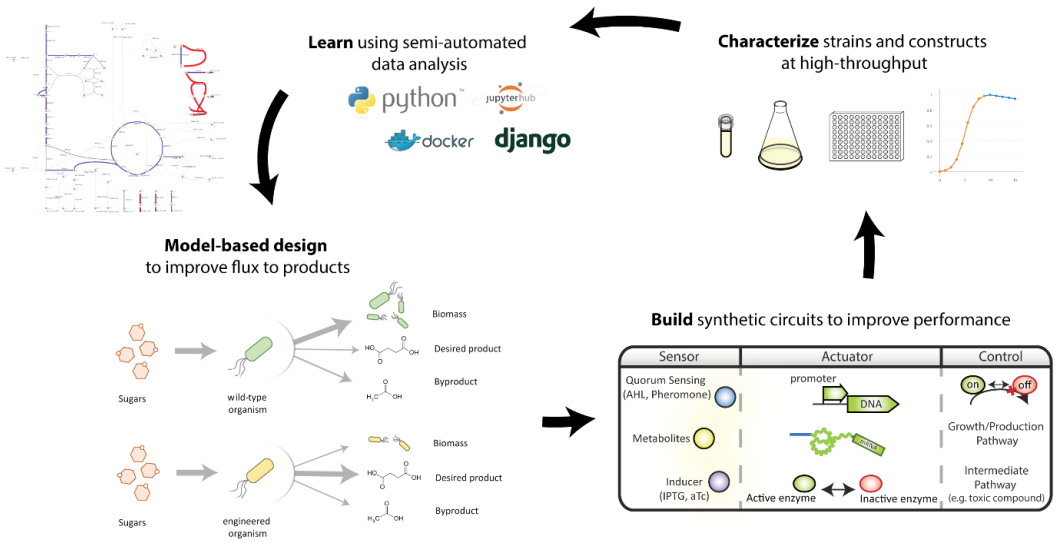

Figure 1: Research overview.

Kaushik Raj Venkatesan
Ph.D. Candidate

B.E. (Hons), 2015, Birla Institute of Technology and Science

Supervisor: Radhakrishnan Mahadevan

Enhancing genetic circuit performance in synthetic biology

Microorganisms have been used to produce several chemicals of industrial and pharmaceutical importance over the past few decades. The advent of metabolic engineering has helped fuel an improvement in the production yields of these chemicals. However, several metabolic engineering strategies result in a growth impediment of the host organism and this results in strains that are not suitable for industrial scale production due to their low productivity.

Dynamic control of metabolism through input regulated switching of gene expression has been proposed as a potential solution to this problem. Advancements in synthetic biology have led to the creation of a device - the genetic toggle switch which is able to switch the cell's phenotype between stable states based on an input. Implementation of toggle switches however, has been limited due to a lack of robustness or very slow switching speed.

We hypothesize that in any synthetic biological circuit, there is an inherent trade-off between the robustness to protein production rates and the response speed of the circuit. My work aims to examine the presence of such a trade-off that a synthetic circuit poses on a cell through experimental evaluation using *E.coli* as a model host and will result in a deeper understanding of the reasons for failure of synthetic circuits. Design guidelines for synthetic biological circuits to minimize the probability of circuit failure will also be proposed.

Research Highlights

Mitacs Globalink Graduate Fellowship, MITACS. (2015-2018)

Ontario Trillium Scholarship, Government of Ontario. (2015-2019)

Research Highlights

T. V. Vuong, B. Liu, M. Sandgren, E. R. Master. (2017). Microplate-based detection of lytic polysaccharide monooxygenase activity by fluorescence-labeling of insoluble oxidized products. *Biomacromolecules,* **18**, 610-616.

T. V. Vuong, M. Foumani, B. MacCormick, R. Kwan, E. R. Master. (2016). Direct comparison of gluco-oligosaccharide oxidase variants and glucose oxidase: Substrate range and H2O2 stability. *Sci. Rep.,* **6**.

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T. V. Vuong *et al.* (2013). Xylo- and cello-oligosaccharide oxidation by gluco-oligosaccharide oxidase from *Sarocladium strictum* and variants with reduced substrate inhibition. *Biotechnol. Biofuels,* **6**.

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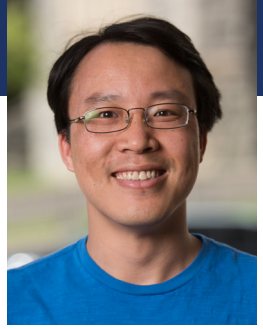


Thu Vuong
Research Associate

B.Biotech., 2000, Hanoi University of Science
M.Biotech., 2004, Flinders University
Ph.D., 2010, Cornell University

Supervisor: Emma Master

Characterization and applications of carbohydrate oxidoreductases

Carbohydrate oxidoreductases could subtract electrons from carbohydrates and donate them to either molecular oxygen or other electron acceptors, or they could transfer one oxygen atom to carbohydrates. Therefore, these enzymes play a key role in derivatizing carbohydrates as well as breaking down lignohemicellulosic materials; as a result, they are being used in biorefinery applications, as well as food, biosensing, biomaterial applications. However, these groups of enzymes are tremendously under-investigated. We have intensively characterized a fungal gluco-oligosaccharide oxidase, expanding its substrate profile while alleviating substrate inhibition. We also fused this enzyme with carbohydrate-binding modules to facilitate its purification as well as food and medical applications. In addition, to help in uncovering new oxidoreductases that modify insoluble lignocellulose surfaces, we have developed methods to monitor the collaborations of lytic polysaccharide monooxygenases with glycoside hydrolases, as well as to screen their activity in insoluble cellulose, providing more insights of their action on biomass. More carbohydrate oxidoreductases are planned to be characterized, providing new tools for industrial applications while expanding our knowledge on their physiological roles in plant-degrading organisms.

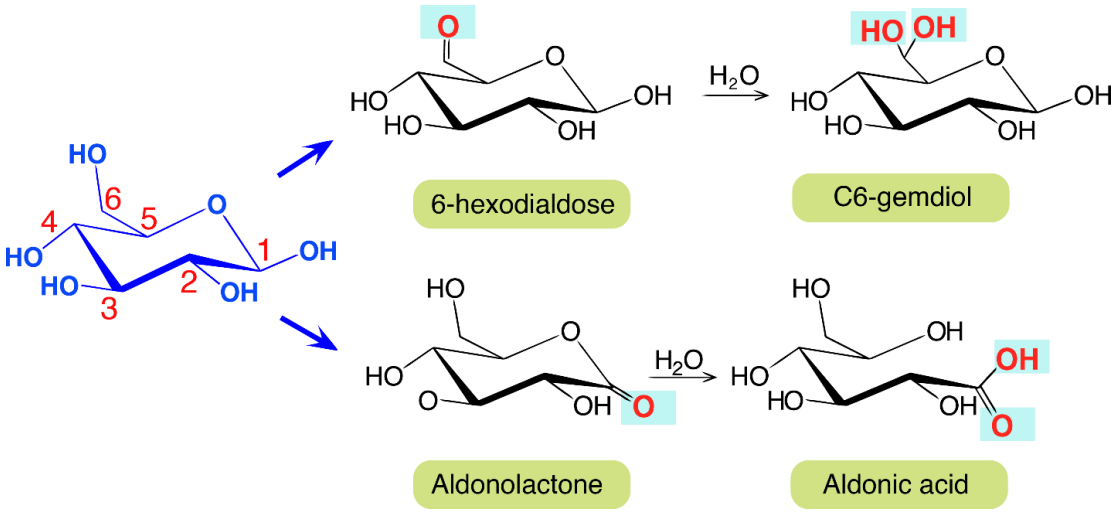
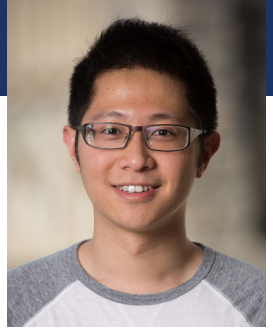


Figure 1: Enzymatic oxidation positions and their corresponding products in aqueous conditions. Oxidation at the anomeric carbons (for instance, by SstAA7A, a gluco-oligosaccharide oxidase from Sarocladium strictum) eventually generates aldonic acid sugars while gemdiols are the final products of oxidations at the C6 position.



Po-Hsiang (Tommy) Wang
Ph.D. Candidate
Supervisor: Elizabeth Edwards

Interspecies nutrient transfer in anaerobic dechlorinating microbial communities

Most microorganisms in the environment live in close association with one another. The importance of studying microbial communities and their interactions is becoming apparent in many fields, including agriculture, bioremediation, human health, waste treatment and industrial biotechnology. Microbes in mixed communities function very differently from microbes in isolated cultures. Complex, yet specific interspecies interactions result in emergent phenotypes not present in pure cultures, and thus microbial communities need to be studied as a whole. Moreover, because of these complex interdependencies, it is often challenging to isolate all the dominating microbes from the communities. Genome-scale metabolic models have been used to gain insights into the molecular mechanisms of individual organisms whose genomes and growth characteristics are known. They are now increasingly being used to help elucidate metabolic interactions between microorganisms at the community level. Thus, my research utilizes a poorly characterized anaerobic subsurface microbial community used for bioremediation of chlorinated solvents, referred to as ACT-3, as the model. The genome of the dechlorinating microbe in ACT-3, *Dehalobacter restrictus* strain CF, has been assembled and annotated via the incorporation of comparative genomic and functional genomic data. However, bioinformatic analyses, while powerful, are often insufficient to reliably predict many metabolic features of an organism because of the considerable number of mis-annotations and hypothetical genes in every genome. Thus, strain CF was isolated and grown in defined medium to study the specific genomic predictions, particularly where annotations were uncertain or inconsistent with the observed phenotypes. The results of the experimental verifications reveal two aspects of the interpretation of genome annotation that can improve the correspondence between bioinformatic predictions and the reality: (i) cofactor availability for corresponding metabolic reactions and, (ii) the potential for enzyme promiscuity to rescue apparently missing pathways, as well as identify essential nutrient interdependencies in anaerobic dechlorinating microbial communities.

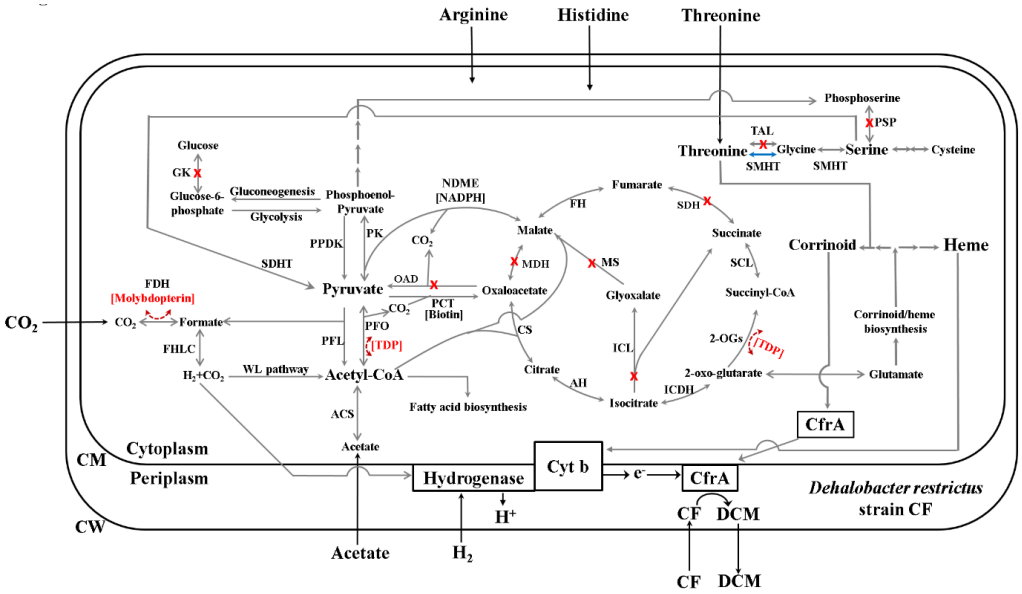


Figure 1: Schematic of the proposed metabolic map of strain CF.

Research Highlights

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P. H. Wang *et al.* (2016). Refined experimental annotation reveals conserved corrinoid autotrophy in chloroform-respiring *Dehalobacter* isolates. *ISME J.*, **11**, 626-640.

Research Highlights

R. Yan, T. V. Vuong, W. Wang, E. R. Master. (2017). Action of a GH115 α-glucuronidase from *Amphibacillus xylanus* at alkaline condition promotes release of 4-O-methylglucopyranosyluronic acid from glucuronoxylan and arabinoglucuronoxylan. *Enzyme Microb. Technol.*, **104**, 22-28.

M. T. Wong *et al.* (2016). Substrate-driven convergence of the microbial community in lignocellulose-amended enrichments of gut microflora from the Canadian beaver (*Castor canadensis*) and North American moose (*Alces americanus*). *Front. Microbiol.*, **7**.

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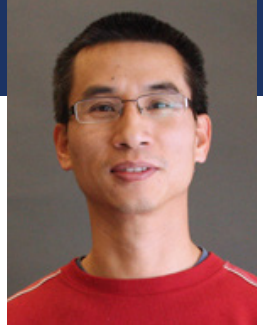


Weijun Wang
Research Associate

B.Sc., 1992, South China Agricultural University
Ph.D., 1997, South China Agricultural University

Supervisor: Emma Master

Enzyme and microbial technology to transform underused biomass to value-added products

Xylan is the second most abundant plant cell wall polysaccharide after cellulose with the great potential for producing renewable fuels, chemicals and materials. Its bioconversion requires enzymes able to efficiently and selectively remove side groups from xylan. Herein, my work is focusing on discovery and design of α ±-L-arabinofuranosidases (GH62/43) and α±-glucuronidases(GH115) for facilitating the recovery and saccharification of xylan. Multiple approaches have been applied in this project, including genomic/metagenomics sequence mining, enzyme structure and functional characterization, and enzyme rational design. Collaborative efforts through BioZone have significantly advanced our understanding of this group of enzymes, but also opened new windows for xylan value-added application.

As a research associate in Dr. Emma Master's Lab, I also lead an industrial collaborative project "Combined enzymatic and mechanical processing of spent ethanol yeast for recovery of high-value bioproducts." Together with the Grober Nutrition, the goal of the project is to develop a novel feed ingredient for young animals.

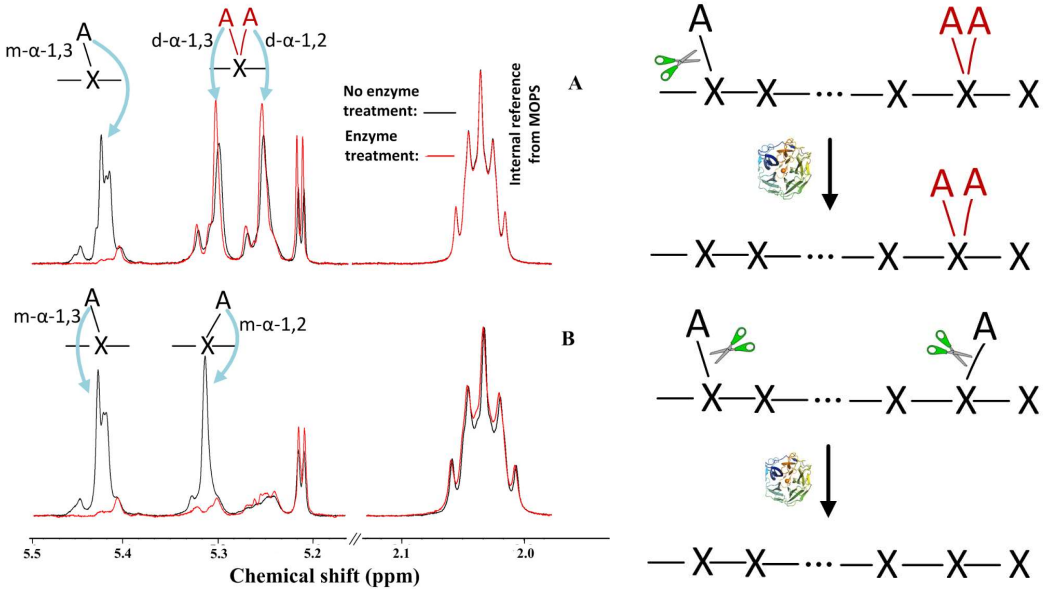


Figure 1: Selectivity towards mono-substituted positions was confirmed by NMR.

Mabel Wong
Ph.D. Candidate

B.Sc., 2007, University of Hong Kong
M.Phil., 2012, University of Hong Kong

Supervisor: Emma Master
Co-Supervisor: Elizabeth Edwards

Bioprospecting environmental lignocellulases

Enzymatic conversion of wood biomass to valuable chemicals and energy is key to establishing a sustainable bio-economy in Canada. Whilst enzyme technologies for the bioconversion of lignocellulose have advanced dramatically in recent decades, the economic viability and diversification of bioproducts require reduction in enzyme costs and new enzyme activities. With a specific focus on wood-degradation, digestive systems of Canadian beaver (*Castor canadensis*), North American moose (*Alces americanus*), and pulp mill anaerobic granules were identified for prospecting microbial lignocellulases. Adapted to the restricted nutritional conditions, the microbial consortia in these niches are evolved to degrade wood via efficient enzymatic actions. Herewith, the proposed project aims to apply a metagenomic approach to characterize the microbial communities involved in bioconversion, and to discover useful wood-degrading enzymes via dual sequence- and function-based search methods. Through serial anaerobic cultivation with wood recalcitrant and degradation inhibitor, key lignocellulolytic microbes have been enriched to express a reservoir of valuable lignocellulases. High-throughput sequencing of the 16S ribosomal RNA gene and metagenomes would help to discern both membership and functional roles of the microbial communities. Based on the annotated polysaccharide utilization loci and key CAZyme families, selected putative lignocellulose-active proteins will be expressed for wood-degrading activities. Concurrently, secretomes from the microbial enrichment cultures will be analyzed via proteomics and assayed for wood-degrading function. Altogether, the combined approaches shall yield a detailed picture of the concerted microbial efforts in wood degradation, and discover novel lignocellulases and non-catalytic proteins that can be applied to pretreat biomass for efficient biorefinery.

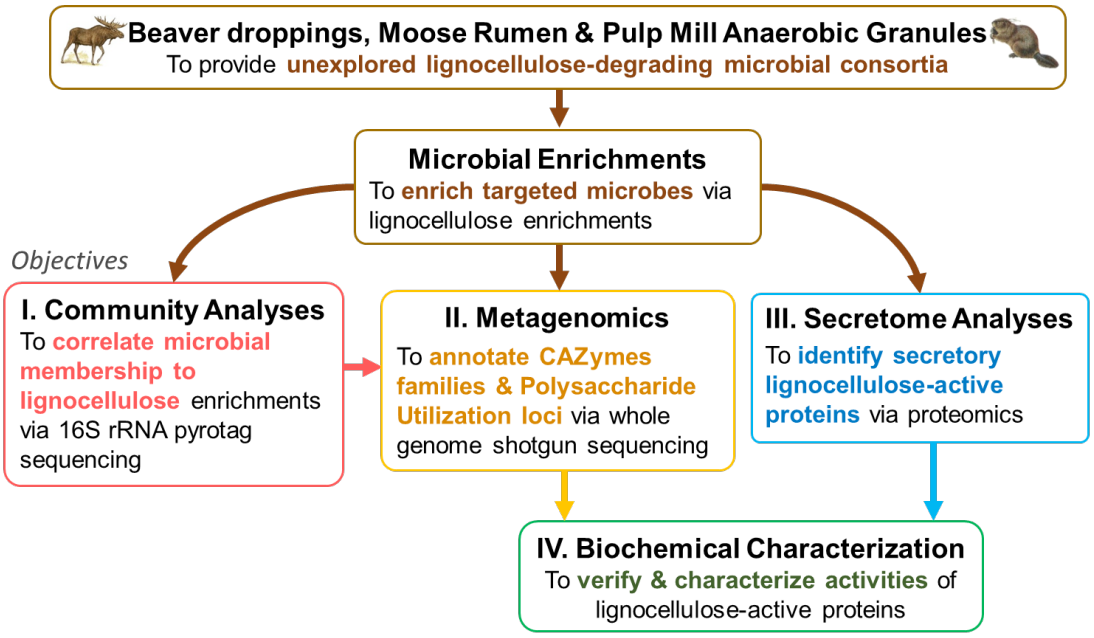


Figure 1: Research overview.

Research Highlights

W. Zhai *et al.* (2017). Arsenic methylation and its relationship to abundance and diversity of arsM genes in composting manure. *Sci. Rep.*, **7**.

M. T. Wong *et al.* (2016). Substrate-driven convergence of the microbial community in lignocellulose-amended enrichments of gut microflora from the Canadian beaver (*Castor canadensis*) and North American moose (*Alces americanus*). *Front. Microbiol.*, **7**.

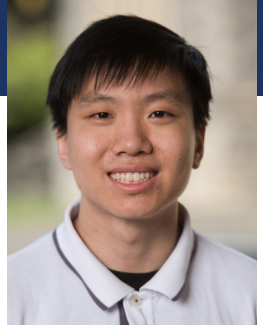
Professor William F. Graydon Memorial Graduate Fellowship, University of Toronto; Dept. of Chemical Engineering. (2016)

BIoZone Recognition Awards - Community Service, BioZone. (2016)

BioZone Graduate Scholarship, BioZone. (2014)

University of Toronto Fellowship, University of Toronto. (2013-2017)

NSERC Canada Graduate Scholarships - Master's Program, NSERC. (2016)



Johnny Xiao
M.A.Sc. Candidate

B.A.Sc., 2015, University of Toronto

Supervisor: Elizabeth Edwards

Heterologous expression in *Clostridium acetobutylicum*

Recent advances in DNA sequencing technology and metagenomics have resulted in a large number of gene sequences that lack functional annotation. The dependency on traditional heterologous expression hosts such as *E. coli* has confined the progress of functional annotation from phylogenetically distant species, since the physiological differences between organisms in distant phyla often fail to express the protein in a functional form. One such protein is a putative anaerobic benzene carboxylase that has been identified in a benzene-degrading *Peptococcaceae* sp., where heterologous expression using *E. coli* was attempted, but failed to yield a sufficiently soluble fraction for biochemical characterization. *Clostridium acetobutylicum* is proposed as an alternative expression host because of its robustness, rapid doubling time, and its phylogenetic proximity to *Peptococcaceae*, thus being more likely to contain the necessary molecular chaperones and cofactors for functional expression of the putative benzene carboxylase. Although systems for genetically modified *C. acetobuylicum* have been developed, work on functional expression of putative genes remains limited. Currently, the putative benzene carboxylase genes have been cloned into several *E. coli-Clostridium* shuttle vectors, but optimized protocols for *C. acetobuylicum* growth, transformation, and expression required for functional expression still need to be developed. Success in this work will provide a benzene carboxylase for functional characterization, and validate the gene as a biomarker in the monitoring of benzene-contaminated sites. The optimized *Clostridium* gene expression system would serve as a convenient expression platform for enzymes naturally expressed by obligate Gram-positive anaerobes and expand the functional annotation of metagenomes from anaerobic environments.

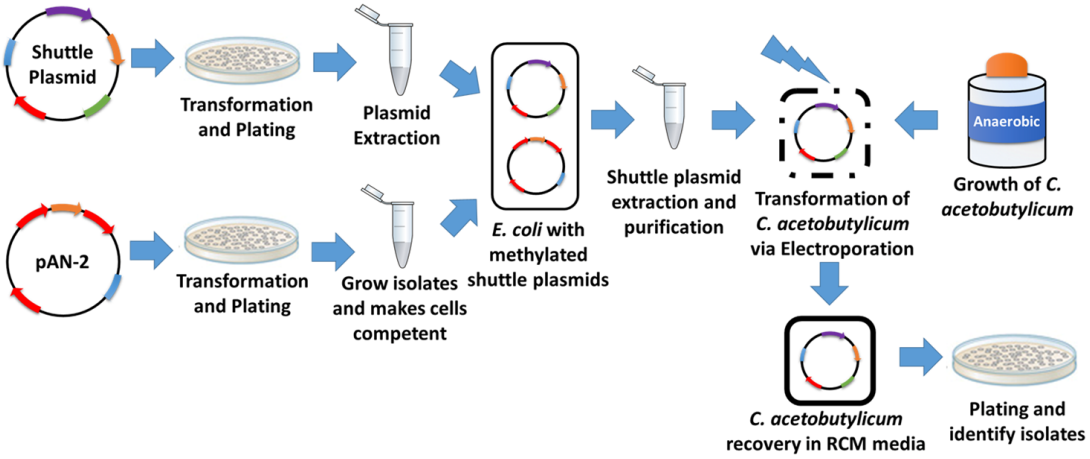


Figure 1: Procedure for transforming C. acetobutylicum. Shuttle plasmids with gene inserts will be constructed and methylated in E. coli before being transformed into C. acetobutylicum using electroporation. (Mermelstein et al., 1992)

Bella Xu
Research Assistant

M.Sc., 2011, Fudan University
M.Eng., 2016, University of Toronto

Supervisor: Alison McGuigan
Co-Supervisor: Penney Gilbert

Development of a novel model to study bi-directional niche interactions in skeletal muscle

In acute skeletal muscle loss like trauma caused by surgery, accidents or recovery from medical conditions involving secondary loss of muscle mass, a complete functional recovery is unlikely to be achieved by innate repair. The interactions between muscle regeneration and inflammation have been assumed for decades, and recently the surprising level of coordination between them has been revealed. However, understanding spatial and temporal links between immune system before and after muscle injury is limited by the lack of effective models to recapitulate complicated muscle environment *in vitro*, while enabling simple stratification and acquisition of data from different cell populations. Here, we describe an engineered model Tissue Roll for Analysis of Cellular Environment and Response (TRACER) that provides a simple strategy to control culture heterogeneity, but simultaneously preserves complex cell-cell interplay that is not possible in traditional co-culture system. Importantly, spatially distinct cell populations can be easily and rapidly isolated on demand for analysis. TRACER has enabled recapitulation of spatial aspects of tumor organization *in vivo*. Now, TRACER is validated to mimic muscle niche interactions based on known knowledge, and then explore bidirectional signaling between muscle progenitors and immune system during muscle tissue maintenance and repair. Identifying the role of inflammation in skeletal muscle tissue will provide new therapeutic targets to improve muscle growth or regeneration following injury.

Research Highlights

B. Xu *et al.* (2016). Patterning cellular compartments within TRACER cultures using sacrificial gelatin printing. *Biofabrication,* **8**.

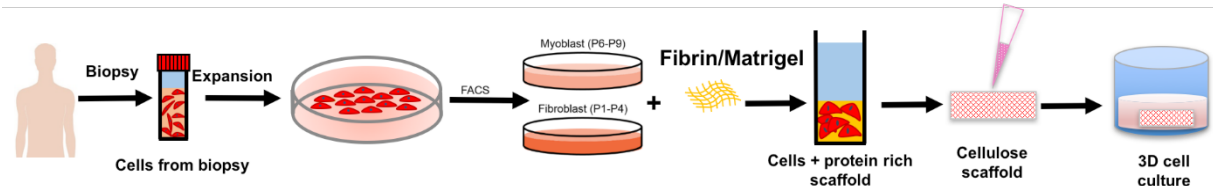
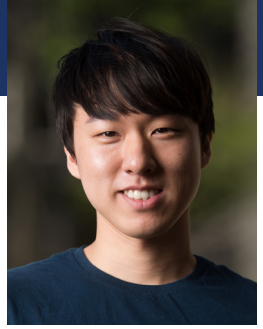


Figure 1: 3D model in vitro *of human skeletal muscle.*



B.A.Sc., 2012, University of Waterloo

Jaehoon (Jason) Ya
M.A.Sc. Candidate

Supervisor: D. Grant Allen
Co-Supervisor: Honghi Tran

Electro-dewatering of pulp and paper mill biosludge

The dewatering of biosludge, the waste generated from the secondary aeration tank of waste treatment processes, has been a major challenge for many pulp and paper mills. The conventional mechanical pressure dewatering method implemented by many industrial mills can only increase the dry solids content up to 4-16% with pure secondary biosludge; thus, the biosludge is usually mixed with primary sludge to noticeably enhance the dewaterability. However, many mills are trying to reduce the release of primary sludge for mainly economic reasons, thereby making the mixing of primary and secondary sludge a less sustainable option in the future.

Electro-dewatering technology based on electro-osmosis can significantly increase the dry solids content of the secondary biosludge. Under the application of an electrical field, the charged ions in the electrical double layer of the sludge particles are drawn towards an electrode, resulting in electro-osmotic flow of water. This flow can enhance the water removal from the biosludge thereby increasing the dry solids content.

Many researchers have studied the electro-dewatering technology on municipal and industrial biosludge; however, only a few studies have examined the effect of the technology on the biosludge produced from pulp and paper mills and similar wood residues. My research, which focuses on the application of electro-dewatering technology on the biosludge produced from pulp and paper mills, will investigate the extent of the dewaterability of electro-dewatering. Other important characteristics of the electro-dewatering technology such as the mixture of different sludges, the energy consumption rate, and the effect of supplementary conditioners will be examined as well.

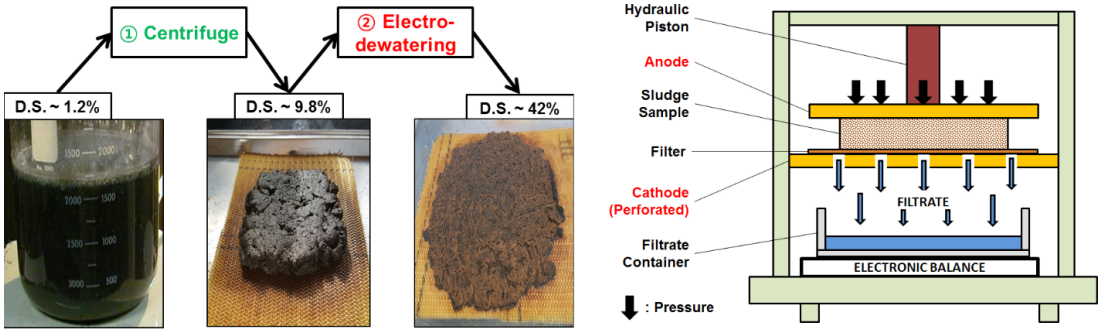


Figure 1: Experimental procedure and the schematic of electro-dewatering.

Ruoyu Yan
Ph.D. Candidate

M.Sc., 2011, University of Western Ontario

Supervisor: Emma Master

Characterizing two novel GH115 alpha-glucuronidases, synergism among accessory xylanases and developing a LC-MS/MS method for characterization of oligomeric sugars

Xylans represent the second most abundant polysaccharide in plant cell walls. Whereas this abundant polysaccharide can be used for the production of renewable energy, chemicals, and materials, it remains comparatively underutilized, mainly due to the complexity of corresponding molecules, whose chemistry depends on plant source as well as process technologies used to separate these components from other cell wall components. Accordingly, the overall objective of my doctoral research project is to harness enzyme selectivity to specify and fine-tune xylan chemistry, to enable broader application of xylans in coatings and as nutraceuticals in food and feed. A main aim is to characterize poorly studied clans of glycoside hydrolase (GH) family 115 α-glucuronidase phylogenies, since GH115 enzymes can selectively remove glucuronic acid/4-O-methyl-D-glucuronic acid (GlcA/MeGlcA) side groups from xylans with high molecular weight.

Research Highlights

R. Yan, T. V. Vuong, W. Wang, E. R. Master. (2017). Action of a GH115 α-glucuronidase from *Amphibacillus xylanus* at alkaline condition promotes release of 4-O-methylglucopyranosyluronic acid from glucuronoxylan and arabinoglucuronoxylan. *Enzyme Microb. Technol.*, **104**, 22-28.

W. Wang *et al.* (2016). Biochemical and structural characterization of a five-domain GH115 α-Glucuronidase from the marine bacterium *Saccharophagus degradans* 2-40T. *J. Biol. Chem.*, **291**, 14120-14133.

D. Jeremic, R. E. Goacher, R. Yan, C. Karunakaran, E. R. Master. (2014). Direct and up-close views of plant cell walls show a leading role for lignin-modifying enzymes on ensuing xylanases. *Biotechnol. Biofuels*, **7**.

Research Highlights

T. Meyer, M. I. Yang, H. N. Tran, D. G. Allen, E. A. Edwards. (2016). Impact of resin and fatty acids on full-scale anaerobic treatment of pulp and paper mill effluents. *Environ. Eng. Sci.,* **33**, 394-403.

M. I. Yang, E. A. Edwards, D. G. Allen. (2010). Anaerobic treatability and biogas production potential of selected in-mill streams. *Water Sci. Technol.,* **62**, 2427-2434.

Minqing (Ivy) Yang
Postdoctoral Fellow

B.A.Sc., 2005, University of Toronto
M.A.Sc., 2008, University of Toronto
Ph.D., 2015, University of Toronto

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Co-Supervisor: Brent Sleep

Groundwater remediation using ZVI

1,1,1-trichloroethane (1,1,1-TCA) had been widely used as an organic solvent, adhesive compound and cleaning agent in many industries before 1989 when it was identified as an ozone-depleting substance in the Montreal Protocol. 1,1,1-TCA can also act as a central nervous system depressant and may cause respiratory tract irritation. The concentration of 1,1,1-TCA in groundwater and soil is regulated.

We are interested in the remediation of 1,1,1-TCA using a combination of biodegradation and chemical reduction with zero valent iron (ZVI). Previous research of ZVI in dechlorination mainly concentrated on nanoscale iron particles, with a focus on chlorinated ethene, such as perchloroethene (PCE) and trichloroethene (TCE). Only a few published papers examined the effectiveness of ZVI in 1,1,1-TCA reduction. Furthermore, the current knowledge of ZVI on the native 1,1,1-TCA degrading community is also limited, although community study can provide insights into the biodegradation process that can help optimize remediation strategies.

In this research, we used coarse ZVI instead of nanoscale ZVI (nZVI), which may have advantages in easier preparation and preservation, as well as lower cost, as compared to nZVI. We are investigating chemical and bio-reduction of 1,1,1-TCA in groundwater in the presence of coarse ZVI, and the impact of ZVI on the native microbial community. This project includes both field study and batch assay study. In the field study, groundwater samples were collected over a nine-month period following iron injection, and the concentrations of chlorinated compounds were examined. Batch assays were set up to study the fate of 1,1,1-TCA under various conditions: sterile conditions with and without coarse ZVI, and active conditions with and without coarse ZVI. Moreover, DNA samples were collected periodically from both the site samples and the batch samples to assess the changes in the microbial communities.

Both field data and batch assays revealed that 1,1,1-TCA was reduced to 1,1-dichloroethane (1,1-DCA), then to mono-chloroethane (CA) at the presence of coarse ZVI. The active bottles without iron also showed the same path, with a lag time but possibly a similar or even faster removal rate. The batch assay study is still ongoing to examine the effect of coarse ZVI on biodegradation. High throughput sequencing and real-time PCR (qPCR) are being conducted to identify and quantify the dechlorinating species present in the native environment (e.g., groundwater).

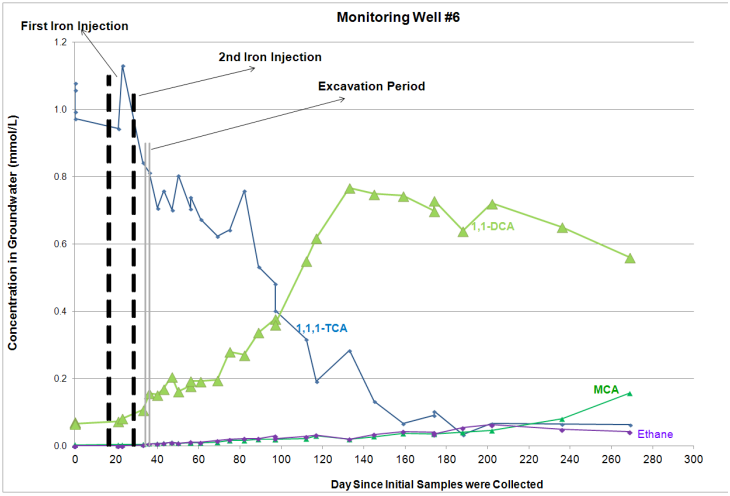

Figure 1: Concentration of groundwater contaminants over time following ZVI injection at monitoring well site.

Mitchell Zak
M.A.Sc. Candidate

Supervisor: D. Grant Allen

Biozone 3-Minute Thesis Competition Winner, Bio-Zone. (2017)

Applications of algal biofilms

Algae possess a variety of potential applications, ranging from biofuel production to wastewater treatment. However, algae cultures have a very low density and therefore most of these applications are economically unfeasible due to high cost of separating water and algae. A possible solution to this problem is to grow algae in the form of a film resulting in a much higher density and reducing separation costs. For this purpose a new type of biofilm photobioreactor called a waveguide, which "leaks" out light allowing for illumination of the bottom side of the biofilm, was developed.

While the knowledge of algae grown in planktonic cultures is quite extensive, there is less information available on their behaviour when they are grown in biofilms. Furthermore, given the new nature of the waveguide, there is little information regarding the applications that it is best suited for. Therefore, it becomes necessary to research the effectiveness of the waveguide as a photobioreactor and how best to utilize it.

For this reason we are currently looking at applying the waveguide in opaque waters where algae cannot typically grow as light cannot penetrate through. One example of this is in the removal of heavy metals from water by biosorption via algae. Also, the removal of nutrients from dark effluents, like in processes such as anaerobic digestion, is another area of interest.

Ultimately, the results of this research should determine which application the waveguide system should be implemented for, allowing for further development and optimization of the waveguide-based photobioreactor.

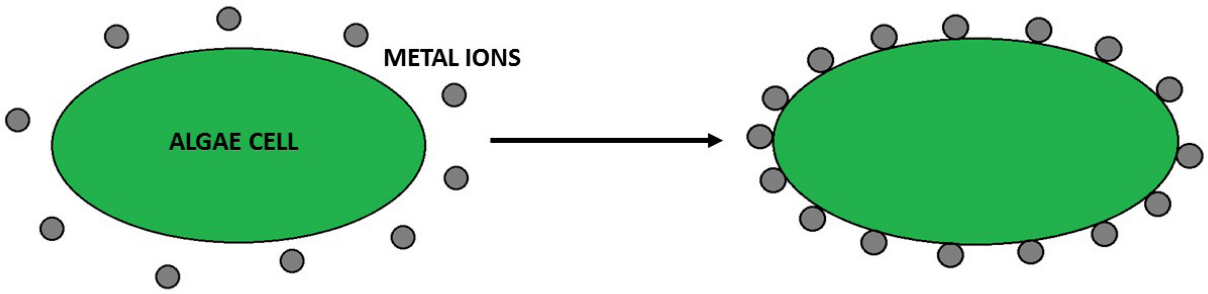

Figure 1: Adsorption of metal ions onto an algae cell wall.

Dan Zeng
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B.Sc., 2011, Xian Jiaotong University
M.A.Sc., 2012, Xian Jiaotong University
Ph.D., 2016, Xian Jiaotong University

Supervisor: D. Grant Allen

Comparative study of triolein transesterification using three supercritical alcohols

Thermodynamic studies have proven particularly useful for predicting the reaction equilibria and evaluating the thermodynamic feasibility of a given process. However, to date, there have been virtually no thermodynamic investigations of the transesterification of triolein with supercritical ethanol or isopropanol. Very few reports are available to thermodynamically justify the selection of methanol as the most suitable alcohol for transesterification with triolein. Thus, an in-depth theoretical study is of great value.

A comparative study of triolein transesterification using three individual supercritical alcohols (methanol, ethanol, and isopropanol) was performed using thermodynamic analysis. The relative properties were calculated by the properties estimation module of Aspen Plus software using the group contribution (UNIFAC) principle for all components, including boiling point, critical parameters, acentric factor, enthalpy, entropy and constant-pressure heat capacity. Chemical equilibria of the three reaction systems were discussed and diagrams of reaction enthalpy, Gibbs free energy and the chemical equilibrium constant as a function of temperature were constructed. The results illustrated that, in the supercritical state, the triolein transesterification reaction proceeds primarily with methanol under proper reaction conditions, but rarely occurs with ethanol or isopropanol. This observation was consistent with the experimental results reported in literature. Our study firstly verified that methanol is the most suitable alcohol for biodiesel production by thermodynamics and provides a reliable method for analyzing analogous reaction systems for biodiesel synthesis with supercritical fluids.

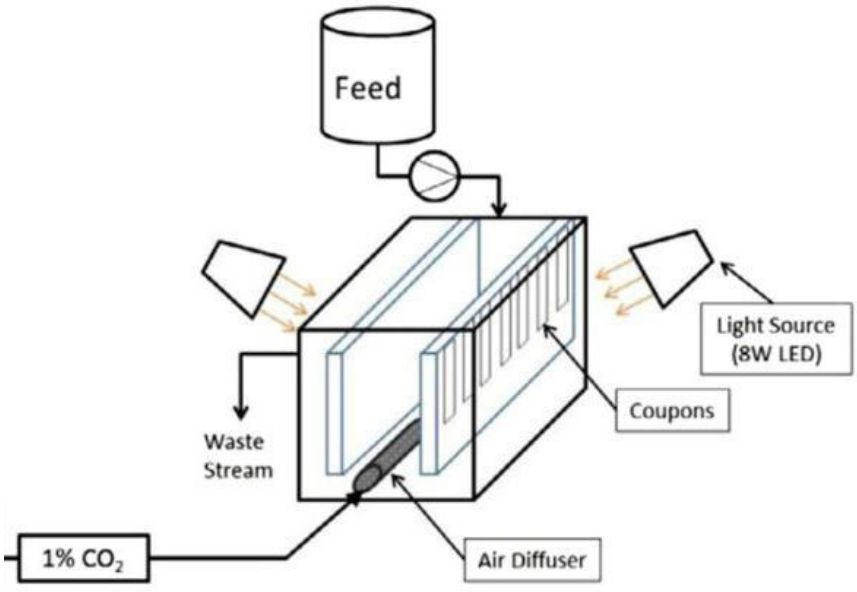

Figure 1: Research overview.

We again wish to thank our many industrial partners, some of whom are shown below, who have supported our ongoing research.

We would like to graciously thank all the public sector agencies who continue supporting BioZone's students, researchers and faculty.

UNIVERSITY OF TORONTO
FACULTY OF APPLIED SCIENCE & ENGINEERING

Report on BioZone

2015 - 2016

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