GRADUATE AND POSTDOCTORAL STUDIES

McGILL UNIVERSITY

FINAL ORAL EXAMINATION
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

OF

ANIL KUMAR PATEL
BIORESOURCE ENGINEERING

MICROALGAE FOR WASTEWATER TREATMENT AND BIOMASS PRODUCTION: A COMPARATIVE ANALYSIS OF GROWTH AND NUTRIENT REMOVAL INCLUDING SHOTGUN PROTEOMICS

Friday August 28, 2015
1:15 PM

MACDONALD STEWART BUILDING, MS2-022
McGill University, Macdonald Campus

COMMITTEE:
Dr. Xin Zhao (Pro-Dean) (Animal Science Department)
Dr. V. Orsat (Chair) (Bioresource Engineering Department)
Dr. M. Lefsrud (Supervisor) (Bioresource Engineering Department)
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Dr. A. Kushalappa (External Member) (Plant Science Department)

Dr. Martin Kreiswirth, Dean of Graduate and Postdoctoral Studies
Members of the Faculty and Graduate Students
are invited to attend
ABSTRACT

Microalgae feedstock and biofuel production can be feasible when a second function is added, such as wastewater treatment. Microalgae differ in uptake of macronutrients and growth within and between species, depending on environmental conditions and perturbation. Identification of promising candidates is onerous and performance in high-density wastewater cultures is poorly understood at the level of cellular pathway. Evolutionary tradeoffs between biomass growth and the stress response of lipid accumulation present a metabolic conundrum that continues to confound yields from mass culturing schemes. This dissertation examined nutrient removal and biomass accumulation in microalgae, and developed shotgun proteomics methods and bioinformatics workflows for the characterization of microalgal physiological states during wastewater processing. A candidate screening of three freshwater and three marine unsequenced species quantified total phosphorus removal and growth as a function of phosphorus loading. Two dual-purpose candidates, *Monoraphidium minutum* sp. and *Tetraselmis suecica* sp., were identified as capable of high rates of phosphorus removal and growth. The sequenced eukaryotic green microalga *Chlamydomonas reinhardtii* was batch cultured at flask and pilot photobioreactor scales to investigate nitrogen and phosphorus uptake and the response of biomass accumulation to wastewater processing. *Chlamydomonas* removed total phosphorus and nitrogen in solution efficiently at both treatment levels and scales. Unexpectedly, treatments did not differ in final biomass for flask cultures. Final biomass was higher in the wastewater treatment at the pilot photobioreactor scale and control cultures were comparable at both scales.

Label-free shotgun proteomic and bioinformatics workflows were developed and used to compare group proteomes at the flask scale in stationary phase. Significant differences in relative protein abundance were found despite no difference in final biomass. Functional analysis showed enrichment of photosynthetic antenna proteins, enzymes related to carbon fixation, and biosynthesis of amino acids and secondary metabolites in wastewater cells. Control cells showed enrichment of enzymes and proteins related to nitrogen metabolism and assimilation, synthesis and utilization of
starch, amino acid recycling, evidence of oxidative stress, and little lipid biosynthesis. Upregulated pathways and genes represent potential targets for genetic improvement in order to develop robust candidate strains suitable for scalable outdoor wastewater treatment and commodity feedstock production.

Findings from candidate screening and scale up of culture volume have implications for future algal and plant research, and high-density biomass production. Proteomic analysis of simulated wastewater processing demonstrated how shotgun proteomics and functional enrichment can be integrated into fundamental biological research concerning algal physiology and genetics, and has potential for bio-process optimization and monitoring. Future investigation is merited using targeted quantitative proteomics, and integration with lipidomic and metabolomic disciplines.

**PUBLICATIONS**


CURRICULUM VITAE

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EMPLOYMENT

MCGILL UNIVERSITY, Department of Bioresource Engineering
09/2010 – 09/2013 Montreal, QC
Lab Manager & Purchasing Manager: Lefsrud Lab

NAVIGANT CONSULTING, INC. (NYSE: NCI), Healthcare Practice
08/2003 – 08/2006 San Mateo, CA
Manager, Business Development: Life Science Division

ALTAVISTA COMPANY (CMGI, INC. funded company), Search engine
07/2000 – 08/2001 Palo Alto, CA
National Account Executive

ECIRCLES.com (CMGI, INC. funded company), Social media
(12/1999 – 05/2000) San Mateo, CA
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