<u>Doctoral of Philosophy in Engineering</u>

Microbial Production of Bio-based Chemicals: A Biorefinery Perspective

Tarek Dishisha

ABSTRACT

A shift from fossil- to renewable biomass feedstock for the emerging bio-based economy requires the development and adoption of new sustainable technologies that are more suited for transformation of biomass components to chemicals, materials and energy. This thesis presents investigations on the development of processes based on industrial biotechnology as a key element for the production of chemicals from agro-/industrial by-products. The chemicals of interest are the ones that could potentially serve as building blocks, platforms, for other chemicals and polymers. Glycerol, a by-product of biodiesel production, was used as a raw material for the production of propionic acid, 3-hydroxypropionaldehyde (3HPA) and 3-hydroxypropionic acid (3HP), while methacrylic acid (MA) was produced from 2-methyl-1,3-propanediol, a by-product of butanediol production. Different strategies to overcome the bottlenecks such as product inhibition existing in the bioprocesses for production of the chemicals were studied.

Fermentation of glycerol to propionic acid was studied using *Propionibacterium acidipropionici*. High cell density cultivations were used to overcome the low production rate caused by slow microbial growth and product-mediated toxicity. Increasing the cell density by immobilization and sequential batch recycling improved the production rates by 2- and 6-fold, respectively, over that obtained using conventional batch. Potato juice, a by-product of potato starch processing, was shown to be a promising, inexpensive nitrogen/vitamin source for the growth of the organism and propionic acid production.

Lactobacillus reuteri was employed as a whole cell biocatalyst for the conversion of glycerol to 3HPA and 3HP in aqueous solution. Production of 3HPA using glycerol dehydratase activity of the cells, limited by substrate inhibition and product toxicity, was performed in a fed-batch mode with *in situ* complexation of the hydroxyaldehyde with bisulfite, and subsequent removal through binding to an anion exchanger. This resulted in increase in production of 3HPA from 0.45 g/g biocatalyst in a batch process to 5.4 g/g in the developed process.

3HP is formed as an oxidation product of 3HPA, however its accumulation as a product of glycerol metabolism in wild-type *L. reuteri* has not been reported earlier. The metabolic fluxes through the glycerol reductive and oxidative pathways were calculated using variable volume fed-batch operation. The glycerol feeding strategies were optimized to yield complete conversion of 3HPA into equimolar mixture of 3HP and 1,3PDO.

MA was quantitatively produced at high purity from 2-methyl-1,3-propanediol by a novel process involving integrated biological and chemical catalysis. Whole resting cells of *Gluconobacter oxydans* were used for selective oxidation of the substrate to the corresponding hydroxycarboxylic acid, which upon dehydration over TiO_2 at 210 ^oC yielded MA. This process offers a potential, significantly greener alternative to the acetone-cyanohydrin process used for MA production, involving highly toxic substrates, large amounts of waste and greenhouse gas emissions.