Le Corps professoral de

Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Monsieur MIAZEK Krystian Michal,

Titulaire d'un diplôme de master engineer in biotechnology, specialization in molecular biotechnology and technical biochemistry,

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 9 février 2018, à 13 heures précises (personne ne sera admis après cette heure),

en l'auditorium du Smart Gastronomy Lab (bât.3),

Passage des Déportés, 2, à 5030 GEMBLOUX.

Cette dissertation originale a pour titre :

« Beech wood *Fagus sylvatica* hydrolysates as feedstocks for *Chlorella* biomass, fatty acid and pigment production ».

Le jury est composé comme suit :

Présidente : Prof. M.-L. FAUCONNIER, Présidente du Département AGROBIOCHEM,
Membres : Dr D. GOFFIN (Promotrice), Prof. A. RICHEL (Copromotrice), Dr M. DELEU, Prof. C. REMACLE, Prof. F. FRANCK, Prof. P. JACQUES, Prof. L. WILLEMS, Prof. A.-L. HANTSON (UMons), Prof. P. VAN CUTSEM (UNamur).

Abstract

This thesis evaluates the possibility of using wood hydrolysates as feedstocks for microalgae growth and production of industrially valuable compounds such as fatty acids and pigments. Moreover, the effect of lignocellulosic hydrolysates, organic substances typically present in lignocellulosic hydrolysates, as well as minor co-products, on growth and accumulation of target compounds in microalgae cultures is described.

Firstly, beech wood dilute-acid (H_2SO_4) hydrolysate as a feedstock for *Chlorella* growth, fatty acid and pigment production, was tested. Hydrolysis of beech wood with the use of sulfuric acid produced a hydrolysate containing numerous organic compounds such as sugars, acetate, phenolics and furans. In order to elucidate the effect of wood hydrolysate on *Chlorella* culture, different components of hydrolysate were tested separately or in the mixture. Amongst compounds tested, glucose and acetate supported *Chlorella* growth, xylose, mannose, galactose, 2-F and HMF were inhibitory, arabinose and rhamnose were neutral. Results of this thesis show that beech wood acid hydrolysate after neutralization with NaOH can strongly improve *Chlorella* growth at lower loadings due to the presence of acetate and glucose, readily consumed in *Chlorella* culture. However, the same hydrolysate added at higher loadings can be inhibitory or even lethal for *Chlorella*. Interestingly, compounds tested separately and identified as inhibitory for *Chlorella* growth, seemed not to be responsible for inhibitory effect of wood hydrolysate. They are either at concentrations too low to cause inhibition (2F, HMF, mannose, galactose) or their inhibitory effect is nullified in the presence of glucose and acetate (xylose, mannose). Neutralized sulfuric acid loadings caused inhibition of *Chlorella* growth. It shows that inorganic compounds added for preparation of wood hydrolysate can also affect microalgae growth. However, neutralized sulfates were only partially responsible for inhibitory activity of wood hydrolysate, showing that wood hydrolysate also contains other substances responsible for growth inhibition. Nevertheless, neutralized wood hydrolysate proved to support *Chlorella* growth during mixotrophic and heterotrophic cultivation, on condition that wood hydrolysate loading is optimized to avoid toxic threshold. This thesis shows that neutralized wood acid hydrolysate can be used as an organic carbon feedstock for microalgae to produce fatty acids and pigments. The addition of 12% wood hydrolysate (Hyd12%) into photoautotrophic culture, improved by nearly 100% fatty acid productivity in comparison to control. Moreover, pigment content in *Chlorella* culture growing on wood hydrolysate in the presence of light, was the highest from all carbon-based profiles. Supplementation of *Chlorella* culture with Hyd12% in dark resulted in fatty acid productivity at comparable level to photoautotrophic control, showing that wood hydrolysate can also become an alternative feedstock for microalgae cultivation in case of lack of light.

Wood acid hydrolysate can serve as a supplement to improve fatty acid and pigment productivity during mixotrophic *Chlorella* cultivation. It can also constitute a source of carbon for fatty acid and pigment production during heterotrophic *Chlorella* cultivation, although it should be taken in consideration that the presence or lack of light was an important factor affecting composition of fatty acids and pigments in *Chlorella* culture, cultivated on a neutralized wood acid hydrolysate.

Secondly, enzymatic beech wood hydrolysate as a feedstock for *Chlorella* growth, fatty acid and pigment production, was tested. Beech wood solids were pretreated with NaOH at high temperature to partially remove xylose and Klason lignin, and enable production of glucose during subsequent enzymatic hydrolysis. A 10% neutralized wood enzymatic hydrolysate containing glucose (TGP-Enz10), was tested on *Chlorella* growth during heterotrophic cultivation and compared with microalgae growth in a medium containing synthetic glucose (TGP). Results show that enzymatic hydrolysate enabled *Chlorella* growth in the dark for biomass, fatty acid and pigment production due to the presence of glucose, although the productivity obtained was smaller, if compared to heterotrophic cultivation in a synthetic TGP medium. Partial growth inhibition and diminished productivity in wood hydrolysate supplemented *Chlorella* culture was due to the presence of neutralized citrate buffer. Neutralized citrate buffer (TGP-Cit10) was found to partially inhibit heterotrophic growth and also strongly suppress mixotrophic growth in *Chlorella* culture. This buffer was also shown to alter fatty acid composition and to slightly affect Chl_{Total}/Car_{Total} ratio during heterotrophic cultivation. Heterotrophic *Chlorella* cultivation with TGP-Enz10 showed that wood enzymatic hydrolysate can constitute a potential feedstock for microalgae cultivation, although the composition of the buffer used during enzymatic hydrolysis should be taken into consideration.

Finally, advantages and disadvantages of using different wood hydrolysates as feedstocks for microalgae cultures, are discussed.

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