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A fermentable glucose-rich stream through the optimal enzymatic hydrolysis of the starch and cellulose fraction of the *Typha domingensis plant* biomass

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AIM AND APPROACH

The macrophyte plant *Typha domingensis* is explored within a framework of integrated plant exploitation. Precisely, we take advantage of both its potential to be used for wastewater treatment, as well as the dual polysaccharide reserve tanks exhibited by the plant, due to its starch and cellulose composition. *Typha domingensis* (cattail) roots and rhizomes demonstrate a high starch content, which is a polysaccharides surplus comparing to herbaceous plants. The available carbohydrates are acquired from two resources: the nonstructural, digestible glucans from starch and the more rigid glucan matrix of structural cellulose [Arai-Sanoh, 2011].

The plant biomass is dried, milled, sieved and classified into three different particle size ranges, with the prospect to investigate the biomass particle size distribution effect on the enzymatic hydrolysis performance. Biomass with particle size between 250-500 μm is homogenised in acetate buffer (T=80^{o}C) before the enzymatic hydrolysis of its starch fraction. Two enzymes from the family of amylases are utilized, a-amylase in the liquefaction process (T=80^{o}C, t=2 h, vortex) and amyloglucosidase in the saccharification process (T=50^{o}C) in an orbital shaker incubator. The free-from-starch biomass fraction (FS) is further treated with dilute aquatic acid solution (2% w/w H_2SO_4) to hydrolyze hemicelluloses (mainly xylans), rearrange the lignin-cellulose structure and reduce crystallinity, in order to deliver a more digestible cellulose fraction. Subsequently, the hydrolysis of cellulose is conducted with the employment of a three-cellulase mixture. Endo- and exo-glucanases and β-glucosidase are utilized in a unique reaction step (T=50^{o}C) in an orbital shaker incubator. The proposed valorization of the entire plant biomass, through the combined starch and cellulose enzymatic hydrolysis, results in a glucose stream of sufficiently high concentration and improved biomass bioconversion index, which generates promising routes for sustainable glucose fermentation to bioethanol and/or other bioproducts.

SCIENTIFIC INNOVATION AND RELEVANCE

Cellulose is the most abundant natural source of carbohydrates and has the potential to serve as a key resource for the production of second-generation biofuels and chemicals, contributing to the partial substitution of petroleum-derived products [Karapatsia, 2017]. Starch resources on the other hand have gained attention for the production of bioethanol, as a 1st generation biofuel. However, such a usage of starch was timely aborted since it generated great ethical issues as the competence of the energy and food sectors for a product tightly related to the human nutrition would signify the onset of a massive starvation problem of the human population around the globe [Martin, 2017]. In the present study, the combination of fermentable sugars hydrolysates, deriving from cellulose and starch fraction of the cattail roots and rhizomes biomass, through a two-stage enzymatic process is investigated in order to maximize the amount of sugars uptake for subsequent fermentation.

Enzymatic hydrolysis is implemented because of the specification of enzymes to produce easily fermentable sugars with no simultaneous formation of toxic compounds that inhibit yeast growth

during fermentation [Mayer, 2015]. The optimization of enzymatic hydrolysis has been studied extensively [Kunamneni, 2005; Sudha, 2018; Garcia 2014]. The major process parameters affecting the efficiency of enzymatic hydrolysis of biomass are the mass loading concentration, the mass ratio of enzymes to biomass and the hydrolysis time, while taking into account the highly determining factors of mass transfer limitations and enzyme activity loss. Thus, high initial substrate can yield high glucose concentrations, which in general improve the overall economics of the subsequent bioprocess steps (i.e., glucose fermentation and product separation) [Jorgensen, 2007], providing though that the mass transfer phenomena allow for sufficient enzyme-substrate contact. On the other hand, an enzyme concentration increase, aiming to digest higher substrate loading and overcome any barriers may appear, is not feasible by economic terms. The optimal hydrolysis conditions in terms of biomass loading and minimum enzyme quantities that increase the biomass conversion and productivity, while confining the mass transfer limitation problem, are investigated through an experimental design.

PRELIMINARY RESULTS AND CONCLUSIONS

The compositional analysis of *Typha* raw biomass exhibited 63.68% carbohydrates (glucans, xylans, arabinoxylans), 15.74% extractives, 17.98% lignin and 2.15% ash on a dry weight base, according to NREL protocol [Sluiter, 2008; 2008a; 2008b]. Starch, cellulose and hemicelluloses constitute 17.67%, 26.55%, 19.46% of the dry biomass, respectively. The effect of biomass loading, enzyme loading and agitation speed, on the efficiency of the enzymatic hydrolysis of both the starch and cellulose plant biomass fractions, was subsequently investigated via a Box Behnken statistical design of experiments with three levels of variation for each one of the studied variables (Table 1). The reaction time, as a constant parameter of the designed experiments, was chosen upon an experimental monitoring of the kinetics of starch and cellulose hydrolysis reactions (Figure 1). The extremely fast reaction rate was evident for both the pure starch and biomass hydrolysis with amylases indicating the completion of the reaction (with a conversion approaching 100%) within less than 24 hours, even for the low enzyme loading (i.e. 50 U/g substrate). On the other hand, the cellulose hydrolysis (either pure, or the respective biomass fraction) proceeds much slower and requires more time to reach a sufficiently high substrate degree of conversion. Thus, the entire study for the statistical investigation of the selected parameters was performed with a time duration of 24 hours for the starch hydrolysis experiments and 48 hours for the cellulose hydrolysis experiments. The derived optimal hydrolysis conditions were implemented for the hydrolysis of the remaining two biomass classes (i.e., the one with particle size below 250 μm and the other with particle size above 500 μm) to demonstrate the effect of particle size distribution. The results of this study provided the control scenario in a preliminary exploration of the enzymatic hydrolytic processes under a fed-batch operating mode in order to make-up for mass transfer imitations and enzyme-substrate accessibility problems.

Table 1. Experimental Design dependent parameters

Independent Variables	**Level -1**	**Level 0**	**Level +1**	**Dependent Variable**
Amylase concentration (U/ g substrate)	30	45	60	Glucose concentration (mg/ml)
Substrate content (% w/v)	6	8	10	
Agitation speed	100	150	200	
Total Runs				15 x 2
Cellulase concentration (FPU/ g substrate)	10	20	30	Glucose concentration (mg/ml)
Substrate content (% w/v)	6	8	10	
Agitation speed	100	150	200	
Total Runs				15 x 2

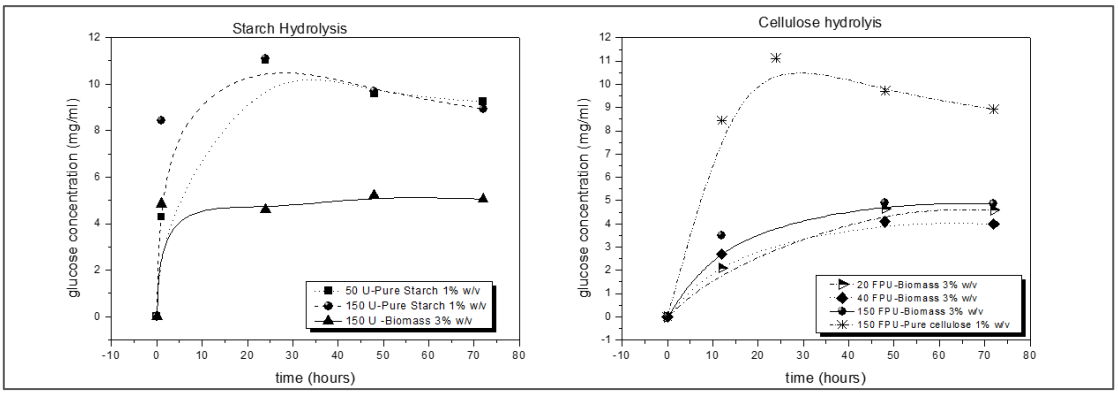

Figure 1: Enzymatic Hydrolysis of pure starch and starch from biomass with different amylases activities - U/g substrate (left diagram) - and cellulose from biomass with different cellulases activities - FPU/g substrate (right diagram).

REFERENCES

Arai-Sanoh Y, Ida M, Zhao R, Yoshinaga S, Takai T, Ishimaru T, Maeda H, Nishitani K, Terashima Y, Gau M, Kato N, Matsuoka M, Kondo M. (2011). Genotypic Variations in Non-Structural Carbohydrate and Cell-Wall Components of the Stem in Rice, Sorghum, and Sugar Vane. Bioscience, Biotechnology, and Biochemistry. 75(6): 1104–12.

Karapatsia A, Pappas I, Penloglou G, Kotrotsiou O, Kiparissides C. (2017). Optimization of Dilute Acid Pretreatment and Enzymatic Hydrolysis of *Phalaris Aquatica* L. Lignocellulosic Biomass in Batch and Fed-Batch Processes. Bioenergy Research. 10(1).

Martín C, Wei M, Xiong S, Jönsson LJ. (2017). Enhancing Saccharification of Cassava Stems by Starch Hydrolysis Prior to Pretreatment. Industrial Crops and Products 97:21–31.

Mayer FD, Gasparotto JM, Klauck E, Werle LB, Jahn SL, Ronaldo Hoffmann, and Marcio Antônio Mazutti. (2015). Conversion of Cassava Starch to Ethanol and a Byproduct under Different Hydrolysis Conditions. Starch - Stärke 67:7–8.

Kunamneni A, Kugen P, Suren S. (2005). Amylase Production in Solid State Fermentation by the Thermophilic Fungus *Thermomyces Lanuginosus*. Journal of Bioscience and Bioengineering. 100(2): 168–71.

Sudha AV, Sivakumar V, Sangeetha V, Priyenka D. (2017). Physicochemical Treatment for Improving Bioconversion of Cassava Industrial Residues. Environmental Progress & Sustainable Energy 37(1): 577–83.

García A, Cara C, Moya M, Rapado J, Puls J, Castro P, Martín C. (2014). Dilute Sulphuric Acid Pretreatment and Enzymatic Hydrolysis of Jatropha Curcas Fruit Shells for Ethanol Production. Industrial Crops and Products. 53: 148–53.

Jørgensen H, Jan Bach K, Claus F. (2007). Enzymatic Conversion of Lignocellulose into Fermentable Sugars: Challenges and Opportunities. Biofuels, Bioproducts and Biorefining. 1(2): 119–34.

Sluiter A, Sluiter J. (2008). Determination of starch in solid biomass samples by HPLC. Technical Report NREL/TP-510-42624. Golden, Colorado.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. (2008a). Determination of structural carbohydrates and lignin in biomass. Technical Report NREL/TP-510-42618. Golden, Colorado.

Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D. (2008b). Determination of extractives in biomass. Technical Report NREL/TP-510-42619. Golden, Colorado.