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Sugar production from cellulosic biosludges generated in a water treatment plant of a Kraft pulp mill

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Abstract

A study on the enzymatic hydrolysis of cellulosic biosludges generated in a water treatment plant of a Kraft pulp mill was carried out. The effect of the operational conditions (cellulase to solid ratio (CSR), liquid to solid ratio (LSR), surfactant concentration (SC) and reaction time), on the hydrolysate composition was evaluated and a set of mathematical models able to predict the glucose and xylose concentrations in the reaction media was proposed.

Using low cellulase charges (8 FPU/g) and high liquid to solid ratios (28–30 g/g), a quantitative conversion of the glucan fraction can be reached in 48 h, although diluted solutions are produced.

However, operating at a cellulase to solid ratio of 12.5 FPU/g, a liquid to solid ratio of 12 g/g and a surfactant concentration of 0 g/L, 74% of the glucan fraction and 67% of the xylan fraction can be saccharified in 34 h, leading to solutions containing up to 27.8 g/L of glucose and 5.4 g/L of xylose.

The results demonstrated that this solid residue shows high enzymatic digestibility and that no pretreatments are needed to enhance the saccharification step. These advantages, along with its negative price, make this solid a valuable raw material for lactic acid production.

Keywords: Biosludges; Cellulose; Waste treatment; Enzymatic hydrolysis; Glucose; Modelling

1. Introduction

Galicia, a region located in the northwest of Spain, is an important wood producer (mainly eucalypt and pine). Most of the eucalypt wood is destined to cellulose pulp production in a local Kraft pulp mill, a kind of factory that needs large amounts of water for the reaction and washing steps. The water streams coming from the washing stages contain lignin and polysaccharides degradation products as well as large amounts of short fibre cellulose, which is not retained in the solid–liquid separation devices.

In order to reduce water consumption, the streams are treated in a water treatment plant and reused. As a result of a biological treatment, several thousand metric tonnes of biosludges (mainly made up of short fibre cellulose and microbial biomass) are generated annually. Nowadays, no added value applications are available for this residue, although it could be used as a raw material for lactic acid production. In comparison to alternative feedstocks, this solid waste shows several advantages, including: (i) high enzymatic digestibility due to its low lignin content and low particle size, (ii) high protein content which could reduce the cost of the nutrients, an important fact due to the expensive nutritional requirements of lactic acid bacteria [1], (iii) negative cost and (iv) environmental benefit because of the reduction of waste volume.

In this context, industrial biosludges are good source of nutrients [2] and have already been used for ethanol production by simultaneous saccharification and fermentation (SSF). Cheung and Anderson [3] and Gao et al. [4] used microbial biomass

Abbreviations: CSR, Cellulase to solid ratio (FPU/g); FPU, Filter paper unit; G, Glucose concentration (g/L); GM, maximum glucose concentration (g/L); IU, International unit on enzymatic activity; LSR, Liquor to solid ratio (g/g); o.d.b., Oven-dry basis; PNPG, *P*-nitrophenyl β-D-glucopyranoside; r_{0G}, Initial rate of glucose production (g/Lh); r_{0TS}, Initial rate of total sugar production (g/Lh); SC, Surfactant concentration (g/L); SSF, Simultaneous saccharification and fermentation; *t*, Reaction time (h); TS, Total sugar concentration (g/L); TSm, Maximum total sugar concentration (g/L); X, Xylose concentration (g/L)

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as a nutrient source, thereby reducing the amount of yeast extract (an expensive product) needed for lactic acid production.

As cellulose is not metabolised by lactic acid bacteria, enzymatic saccharification of this polysaccharide has to be done before fermentation. However, until recently, the cost of enzymes was one of the most significant drawbacks in this kind of processes. In the period of 2001–2005, Novozymes and national renewable energy laboratory (NREL) reduced 30 times the cost of the enzymes needed to produce fuel ethanol from biomass waste. This means that the cost of enzymes may no longer be the most important drawback and that efforts should now be focused on searching for cheaper raw materials and on developing less expensive physicochemical pretreatments. In any case, reduced enzyme consumption is always important from an economic standpoint and the addition of surfactants like Tween 80 has been reported to enhance enzymatic saccharification, thereby reducing the enzyme charge [5]. On the other hand, Eriksson et al. [6] found that the main obstacle in the enzymatic conversion of lignocellulose is the adsorption of significant amounts of enzyme on substrate; a situation that also could happen in the experimental system considered in this study.

This paper deals with the enzymatic saccharification of industrial biosludges generated in a local Kraft pulp mill to produce high sugar-containing solutions. Mathematical models assessing the effect of the operational conditions (cellulase to solid ratio (CSR), liquid to solid ratio (LSR) and surfactant concentration (SC)) on the kinetics of sugar production were developed and optimal operational conditions were selected.

2. Materials and methods

2.1. Raw material

Samples of cellulosic biosludges were collected weekly in a local Kraft pulp mill (ENCE, Lourizán, Pontevedra). Due to their high moisture content (75%) and in order to avoid their degradation, all samples were submitted to a drying stage at 60 °C for 24 h. Then, dried samples were mixed to obtain a representative lot and stored at 4 °C in a refrigerator. Aliquots from the homogenised lot were employed in experiments and an aliquot was used to determine its composition following the methodology described below. Table 1 shows the composition of the residue.

Table 1
Composition of raw material

Fraction	Content (%, o.d.b.)
Glucan	43.9
Xylan	9.4
Protein	22.5
Ashes	6.4
Other fractions (by subtraction)	17.8

o.d.b.: oven-dry basis.

Table 2
Experimental variables involved in the study

Variable	Definition and units	Nomenclature	Value or range
Fixed	Temperature in enzymatic hydrolysis (°C)	T	48.5
	pH in enzymatic hydrolysis	pH	4.85
	Citric acid-sodium citrate buffer concentration (N)		0.05
	Betaglucosidase activity to cellulase activity ratio (IU/FPU)	BCR	5
	Final reaction time in enzymatic hydrolysis (h)	t	48
Independent	Cellulase to solid ratio in the enzymatic hydrolysis (FPU/g)	CSR or x_1	5–20
	Liquor to solid ratio in the enzymatic hydrolysis (g/g)	LSR or x_2	12–30
	Surfactant concentration (g/L)	SC or x_3	0–5
Dependent	Maximum glucose concentration of enzymatic hydrolysates (g/L)	Gm or y_1	
	Initial rate of glucose production (g/Lh)	r_{0G} or y_2	
	Maximum total sugar concentration of enzymatic hydrolysates (g/L)	TSm or y_3	
	Initial rate of total sugars production (g/Lh)	r_{0TS} or y_4	

2.2. Enzymatic hydrolysis

Enzymatic hydrolysis assays were carried out at 48.5 °C in Erlenmeyer flasks with orbital agitation (150 rpm) using commercial enzymes. The pH was kept at 4.85 using 0.05N citric acid–sodium citrate buffer. Commercial enzyme concentrates ("Celluclast 1.5 L" cellulases from *Trichoderma reesei* and "Novozym 188" β-glucosidase from *Aspergillus niger*) were kindly provided by Novozymes (Madrid, Spain). The cellulase activity of "Celluclast 1.5 L" concentrate (lot number: CCN03060) was measured by the filter paper assay [7] and the activity was expressed in terms of filter paper units (FPU). The β-glucosidase activity of "Novozym 188" concentrate (lot number: DCN00206) was measured by the PNPG assay [8] and reported as international units (IU). Table 2 shows the values for the operational variables employed in this study. The cellulase activity corresponded to usual values for these kinds of processes, whereas an excess of β-glucosidase activity was employed in order to prevent the accumulation of cellobiose. Due to its high microbial biomass content, a stronger sterilisation step was needed (40 min at 121 °C) before enzyme addition. At given reaction times (0, 2, 6, 10, 24, 36 and 48 h), samples were withdrawn from the reaction media, centrifuged, filtered and analysed by HPLC (see below).

2.3. Analytical methods

Moisture and ash contents were determined according to the ISO 638:1978 and ISO 776 methods, respectively. Elemental nitrogen determination was carried out by means of a Thermo Finnegan flash EATM 1112 analyzer using 130 and 100 mL/min