Integrated enzymatic pretreatment and hydrolysis of apple pomace in a bubble column bioreactor

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Introduction

Lignocellulosic material is the most abundant source of not exploited biomass, mainly composed of three polymers: cellulose, hemicellulose and lignin. **Agriculture food processing wastes (AFWs)** are potential lignocellulosic material for biorefinery processes being economic and eco-friendly [1]. **Apple pomace** is an AFW representing the main by-product of fresh apple processing aimed at the production of juice, cider, purees and jams [2]. This residue accounts for 25-35% of the dry mass of apple [3]. Due to its great availability, apple pomace is a good candidate to be used in a biorefinery process. **Pretreatment of** this feedstock is necessary to facilitate the release of fermentable sugars during enzymatic hydrolysis. New pretreatment methods based on use of **laccases** were proposed in the last years [ref??]. The process consists in the depolymerization of lignin catalyzed by laccase: at the end greater exposure of carbohydrates polymers is achieved, facilitating their hydrolysis by cellulases. Substrate specificity, mild operating conditions and no inhibitors production are the main advantages of this enzymatic pretreatment. The use of **bubble columns** as bioreactors was investigated in many fermentative/enzymatic processes. The wide application area of bubble column reactor is due to the advantages of this system with respect to other reactors: simple design, excellent heat and mass transfer, low operating costs due to lack of moving parts [4].

Aim

The aim of the study was to determine the effect of **enzymatic delignification** of apple pomace in a bubble column bioreactor and to test the integration of both enzymatic delignifcation and cellulose hydrolysis through sequential operations. Different parameters such as **biomass loading, laccase loading and air flow rate** were investigated to assess the **optimal pretreatment conditions** allowing to obtain the higher amount of fermentable sugars. Enzymatic hydrolysis experiments were also carried out after pretreatment at fixed conditions to maximize the release of reducing sugars from apple pomace. Moreover, a **sequential enzymatic pretreatment and hydrolysis** of apple pomace in the bubble column reactor was carried out to asses the feasibility of the integrated process.

Materials & Methods

BIOREACTOR SET-UP. The cylindrical bubble column (Figure 1) was made of glass (0.4 m height, 0.034 m ID) and was equipped with a water jacket connected to a thermostatic bath. Agitation of the suspension in the column was provided by gas bubbles of air injected at the column bottom through stainless steel tube (0.001 m ID). Air flow rate was controlled with a rotameter. The air stream was water saturated in a humidifier to prevent solvent evaporation in the bioreactor. The gas hold-up (ε_G) in the column was assessed according to Eq. (1), where H0 is the height of the stagnant liquid, and H the height of the liquid in the bubble column at a fixed gas flow rate (from 0 to 120 nL/h).

$$\varepsilon_G = \frac{H - Ho}{H} \qquad (1)$$

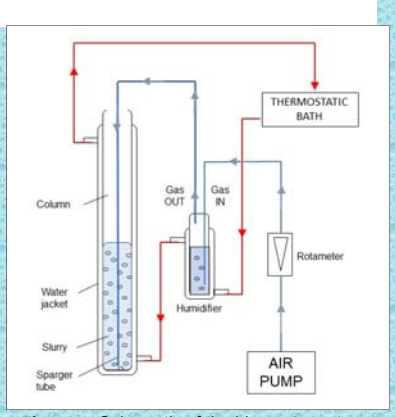


Figure 1. *Schematic of the bioreactor set-up.*

FEEDSTOCK. Apple pomace was provided by the Instituto Tecnologico Agrario Castilla y Leon (ITACyL). The biomass was oven dried at 50 °C until a constant weight was achieved. Dry apple pomace was sieved and particles with a diameter in the range of 0.5-1.0 mm were used for the experiments.

OPTIMIZATION OF THE ENZYMATIC PRETREATMENT. The effects of **biomass loading (BL), laccase loading (LL) and air flow rate (AFR)** on the enzymatic pretreatment of apple pomace in the bubble column were investigated. BL, LL and AFR ranged between 5 and 15 %w/v, 10 and 50 U/gbiomass, 20 and 100 nL/h, respectively. All tests were carried out in 0.1L 0.1 M sodium citrate buffer (pH 5) at 28 °C for 24 h. Enzymatic hydrolysis of pretreatment biomass was carried out in glass bottles at a fixed BL of 10 % w/v. Biomass was added to 0.03L 0.1 M sodium citrate buffer (pH 4.8) at 50 °C with 1 FPU/gcellulose and mixed with a rotary shaker at 180 rpm for at least 72 h. Samples were taken for the analysis of reducing sugar.

ENZYMES. Laccase used for the pretreatment of apple pomace were kindly provided by Biopox s.r.l. (Naples, Italy). Laccase activity was assayed according to a colorimetric assay by using ABTS as substrate. Enzymatic hydrolysis of pretreated apple pomace was carried out using commercial cocktail of hydrolytic enzymes CellicCTec2® (Novozymes, Denmark).

INTEGRATED ENZYMATIC PRETREATMENT AND HYDROLYSIS. The operating conditions giving the best performances in terms of sugar release from otpmization study were adopted to carry out sequential enzymatic pretreatment and hydrolysis of apple pomace in bubble column reactor. After the first step of optimized pretreatment with laccase the hydrolysis was performed at …

ANALYTICAL METHODS. The composition of raw and pretreated apple pomace was determined according to the NREL protocol [5]. Reducing sugars in the liquid phase were quantified by means of 3,5-DNS method [ref?].

Results & Discussion

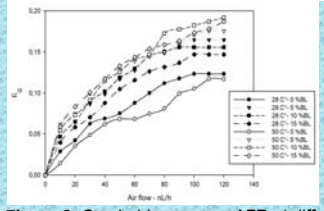
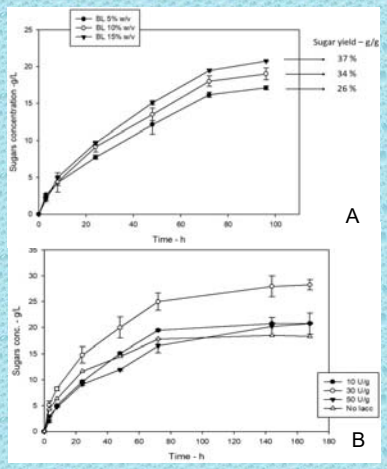


Figure 2 reports the **effects of solid loading and temperature on gas hold-up at different AFR**. The presence of biomass in the solution provided an increase of gas hold-up at fixed temperature with respect to the liquid-gas system. Increase of temperature had opposite effects on ε_g depending on the absence or the presence of biomass. At 28°C the increase in BL provided a decrease in ε_g, at 50°C negligible effect of BL on ε_g was observed.

Figure 2*. Gas hold-up versus AFR at different temperatures and BL.*

The increase of BL (Figure 3A) during the pretreatment causes an increase of sugars release during enzymatic hydrolysis probably due to laccase deactivation by bubble breakage at low BL. The increase of LL up to 30 U/$g_{biomass}$ improved subsequent saccharification (Figure 3B). Increase of AFR provided good mixing up to 60 nL/h (Figure 3C). Higher AFR provided bigger bubbles and likely indirect laccases deactivation due to bubble breakage. **Optimized conditions for laccase pretreatment were: BL…, LL …., AFR …**



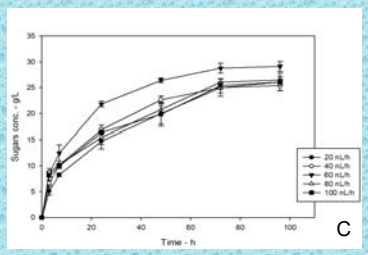
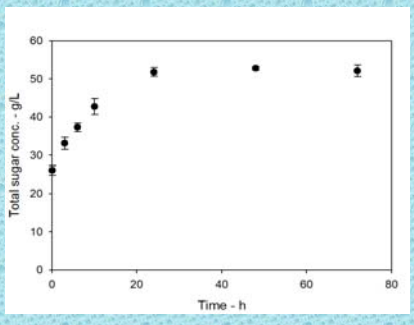


Figure 3*. Hydrolysis profiles of apple pomace pretreated with laccase at different BL (A), LL (B) and AFR (C).*

Table 1 reports the composition of apple pomace after pretreatment with the optimized parameters (Figure 3). From the comparison with control (without laccase), it can be noted that an increase of carbohydrates content was obtained. Moreover, a lignin decrease, corresponding to 16 % delignification, was also achieved.

Table 1*. Composition of apple pomace on dry weight basis [ref?].*

	BL	LL	AFR	Recovery	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Lignin reduction (%)
Raw	/	/	/	/	25.3 ± 0.4	11.3 ± 0.6	17.0 ± 0.2	/
Control	15	/	60	58.9 ± 2.0	31.9 ± 0.8	18.2 ± 0.1	26.7 ± 0.2	/
Pretreated	15	30	60	60.8 ± 0.8	36.4 ± 0.4	19.5 ± 1.3	23.4 ± 0.5	16



A **sequential pretreatment and enzymatic hydrolysis in the bubble column** was carried out at the optimized conditions for laccase delignification steps. The glucose production rate increased in the first 24 h (Figure 4) probably due to cellulases deactivation by bubble breakage and by inhibition of phenolic compounds released during laccase pretreatment. After 24 h a maximum total sugars concentration of 51.8 g/L was reached, corresponding to yields of 0.34 $g_{sugar}/g_{raw\ biomass}$ and 0.61 $g_{sugar}/g_{sugar\ available}$.

Figure 4*. Time course of sugars release in bubble column after optimization of both laccases pretreatment (30 U/gbiomass, 15%w/v biomass loading, 60 nL/h, 24 h) and Cellic CTec2® hydrolysis (60 nL/h; 20 FPU/$g_{cellulose}$).*

Main Remarks

- Gas hold-up was affected by both temperature and biomass loading.
- Biomass loading, laccase concentration and air flow rate affected laccase pretreatment of apple pomace. Air flow rate influenced enzymatic hydrolysis rate (data not shown).
- Sequential enzymatic pretreatment and hydrolysis of apple pomace was successfully carried out in the bubble column bioreactor.

Acknowledgments

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