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Research paper

Pre-treatment and enzymatic hydrolysis of lettuce residues as feedstock for bio-butanol production

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ABSTRACT

The residue of the fresh-cut vegetable packaging process is a major environmental concern for food industries. Actually 50% of the processed vegetables must be disposed of. But the lettuce fraction sorted and deemed unsuitable to be bagged may be used as feedstock for butanol production because it is rich in sugars. In this work acetone butanol ethanol (ABE) was produced from enzymatic-hydrolysed *Lactuca sativa* leaves. The lettuce leaves were first pre-treated with NaOH and then hydrolysed. The NaOH concentration was increased up to 200 kg m^{-3}, Cellic CTec 2 (Novozymes) was used for the hydrolysis. The sugars contained in the biomass hydrolysate were fermented in batch cultures of *Clostridium acetobutylicum* DSMZ 792. The fermentation process was characterized in terms of sugar conversion and ABE production. The hydrolysate of the NaOH-pre-treated lettuce contained glucose and xylose (about 50/50). Under optimal conditions the sugar concentration after the enzymatic hydrolysis was 19.5 g L^{-1}, the ABE concentration was 1.44 g L^{-1} and the butanol concentration was 1.1 g L^{-1}. Some tests were also carried out with synthetic mixtures of glucose and xylose to investigate the effects of the alkaline pre-treatment on the fermentation process.

1. Introduction

The availability of feedstock for Acetone-Butanol-Ethanol (ABE) fermentation is a key to the economic success of the biotechnological route to produce bio-butanol [1], [2]. Agro-food industry residues are particularly interesting as renewable feedstock because they are abundant and un-competitive with food sources. The fresh-cut vegetable industry is growing fast in Europe. The value of the European fresh-cut fruit and vegetable market is about 3.4 billion euros. Salads account for 62% of the market volume in Europe, other vegetables 31% and fruit 7%, respectively [3]. The UK is the market leader in Europe with one third of the total continental fresh-cut fruit and vegetable consumption: about 480,000 t for year. In Italy the fresh-cut production is around 100 kt yr^{-1} [4]. The residues from the fresh-cut industries are a cause of concern because as much as 50% of the raw processed vegetables are deemed unsuitable and their disposal as waste is very expensive [5], [6]. Agro-food residues can be classified as a lignocellulosic biomass as they are characterized by the high crystallinity of cellulose and the presence of lignin. The lignin content in the agro-food residues ranges between 0.2% (dry matter) for orange peel and 33.8% (dry matter) for peanut shell [7]. Because of the presence of lignin a pre-treatment step is necessary before the hydrolysis of the carbohydrates.

Alkaline pre-treatment is one of the most promising technologies. By this process the lignin-cellulose structure is fragmented and the cellulose crystallinity is reduced [8]. There is a great deal of literature on the NaOH-pre-treatment of several agro-industrial residues but to the Authors' knowledge, no study on lettuce has yet been published. After the NaOH-pre-treatment and enzymatic hydrolysis, a mixed sugar stream (mainly glucose and xylose) can be obtained. The sugar fraction ranges between 0.16 g g^{-1} for rice straw [9] and 0.9 g g^{-1} for corn cob [10] depending on the residue. Based on these findings the agro-industrial residues seem to be a promising feedstock for the production of bio-butanol. Furthermore, it is well known that the *Clostridia* strains are able to

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metabolize hexose and pentose sugars and to produce butanol [11].

This paper reports the results of bio-butanol production by fermentation of a hydrolysate of *Lactuca sativa*. Alkaline pre-treatment at different NaOH concentrations was carried out. Then the lettuce was enzymatically hydrolysed to evaluate the pre-treatment efficiency. The resulting hydrolysates were fermented by *Clostridium acetobutylicum* DSMZ 792 for ABE production. The effects of pre-treatment on fermentation were assessed in terms of sugar conversion and solvent production rates as compared to the fermentation of a synthetic medium containing a mixture of simple sugars.

Fig. 1 shows the synoptic diagram of the ABE process using lettuce as feedstock: biomass drying, NaOH pre-treatment, enzymatic hydrolysis of the solids recovered, and ABE fermentation. The lettuce was homogenized and then oven-dried. The dried lettuce was reduced to powder and pre-treated with NaOH. The solid particles collected after the NaOH-pre-treatment were used for enzymatic hydrolysis. The sugar-rich solution produced by the enzymatic hydrolysis was used as substrate for the ABE fermentation process.

2. Materials and methods

2.1. Raw materials

The lettuce tested (*Lactuca sativa*) came from farms in Southern Italy in 2014. The ripe lettuce was harvested, the external leaves were removed according to the procedure adopted in the 'fresh cut' market, stored in a refrigerator for two days and further processed for the tests. The sampled lettuce leaves were homogenized by means of a mixer for few seconds. The produced lettuce pulp was oven-dried at 40 °C, milled to reduce the grain size down to 1 mm and stored in sealed plastic bags at room temperature.

The untreated biomass and the NaOH pre-treated biomass were characterized in term of glucan, xylan and lignin composition according to the standard NREL protocols [12].

2.2. Alkaline pre-treatment

The dried lettuce powder was treated in 100 cm^3 bottles equipped with screwed caps. The dried lettuce powder was soaked in NaOH aqueous solution. The NaOH concentration was set between 4 kg m^{-3} and 200 kg m^{-3}. The powder was dispersed in the alkaline solution: 16 cm^3 of solution was used per gram of solid mass. The suspension was kept in autoclave (VAPORMATIC 770) for 30 min at 121 °C according to the procedure suggested by Gao et al. [10] for corncobs. The biomass was separated from the liquid phase (black liquor) by centrifugation at 5320 RCF for 10 min. The biomass was washed with distilled water until pH 7 was reached, then it was oven-dried at 40 °C and stored to be used for enzymatic hydrolysis. Typically, the solid phase was stored for less than a day.

The black liquor was characterized in terms of sugars (glucose, xylose, arabinose, mannose) and inhibitors (HMF, furfural and acetic acid).

2.3. Enzymatic hydrolysis

The enzymatic hydrolysis was carried out according to the procedure proposed by Gao et al. [10] adapted to the lettuce powder. The enzyme was Cellic CTec 2 (Novozyme) provided as a stock solution at 160 FPU cm^{3-1}. The hydrolysis was carried out in a 4 kg m^{-3}sodium citrate buffer (pH 4.8) supplemented with 80 mm^3 tetracycline and 60 mm^3 cycloheximide to prevent microbial contamination during enzymatic digestion.

The hydrolysis was carried out in 100 cm^3 glass bottles incubated at 50 °C and kept under gentle agitation on a rotary shaker (Minitron Incubator Shaker-Infors HT), 180 rpm for 60 h. The enzyme stock solution to biomass ratio was set at 180 mm^3 g^{-1}. The ratio between the dry matter mass and buffer volume was set at 2% ($w \cdot v^{-1}$).

The enzymatic broth was sampled at pre-set times, centrifuged, filtered and analysed to measure the sugar concentration. All the hydrolysis tests were carried out in duplicate.

2.4. Microorganism and medium

Clostridium acetobutylicum DSMZ 792 was supplied by DSMZ. The stock cultures were reactivated according to the DSMZ procedure. The reactivated cultures were stored at −80 °C. The thawed cultures were inoculated into 12 cm^3 of synthetic medium containing glucose (30 g L^{-1}) and yeast extract (YE) (5 g L^{-1}) in 15 cm^3 Hungate tubes (pre-cultures). The cells were grown under anaerobic conditions for 48 h at 37 °C, then they were transferred to fermentation bottles.

The fermentation medium consisted of 5 g L^{-1} YE, 2.5 g L^{-1} NH_4Cl, 0.25 g L^{-1} KH_2PO_4, 0.25 g L^{-1} K_2HPO_4 and mineral solution (0.20 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.01 g L^{-1} $MnSO_4 \cdot H_2O$, 0.01 g L^{-1} $FeSO_4 \cdot 7H_2O$). The carbon source was the enzymatic hydrolysate of the NAOH pre-treated lettuce.

Some fermentation tests were also carried out with reference sugars (glucose and xylose) to mimic the composition of the biomass hydrolysates. Two sugar compositions were investigated: 10 g L^{-1} glucose, 10 g L^{-1} xylose; and 5 g L^{-1} glucose, 5 g L^{-1} xylose.

2.5. ABE fermentation

The fermentation tests were performed in 100 cm^3 glass bottles filled with 40 mL of medium, the pH of the medium was 6.5 after addition of 400 kg m^{-3}NaOH. The bottles were sparged with nitrogen (technical grade) to create anaerobic conditions. The bottles

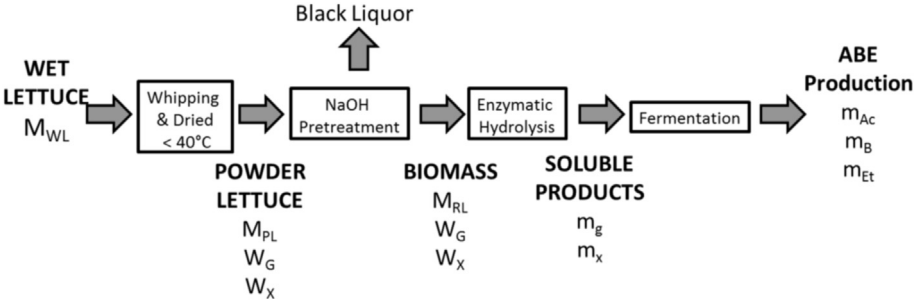

Fig. 1. Flow diagram of the ABE production process from lettuce residues as feedstock (M_{WL} - wet lettuce mass, M_{PL} - powder lettuce mass, M_{RL} -recovered lettuce mass, W_G - glucan fraction of the solid, W_X - xylan fraction of the solid, m_g - glucose mass, m_x - xylose mass, m_{Ac} - acetone mass, m_B butanol mass, m_{Et} - ethanol mass).