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Lactic acid production from recycled paper sludge: Process intensification by running fed-batch into a membrane-recycle bioreactor

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ABSTRACT

Production of lactic acid from recycled paper sludge by simultaneous saccharification and fermentation (SSF) has previously been implemented under a pulsed fed-batch mode. However, lactic acid concentrations above 58 g L^{-1} inhibit cultivation of the *Lactobacillus rhamnosus* strain used. Thereby, the present work targeted process intensification by running it into a membrane-recycle bioreactor, providing product removal together with reuse of enzymes and bacterial cells.

A shear-enhanced flat sheet cross-flow filtration system was built, working properly with the high-solids concentration suspension. Based on product inhibition and solids concentration constraints, a model was proposed for operation of the membrane-recycle bioreactor. SSF should be run in batch mode into the fermentor for 48 h, and then switch-on recirculation through the filtration module, with pulsed fed-batch addition of recycled paper sludge.

This innovative approach can be applied to improve other SSF processes dealing with high-solids concentrations, towards cost-effective lignocellulosic feedstock biorefineries.

1. Introduction

Lactic acid (LA)[1] is a high value and versatile chemical with a rapidly expanding market as precursor of polylactic acid (PLA),[2] a biodegradable and biocompatible polymer with increasing application as substitute of petrochemical-based plastics [1]. High optical purity is a prerequisite for synthesis of the homopolymers poly-L-LA and poly-D-LA, which form regular structures in a crystalline phase contrarily to the amorphous material resulting from copolymerization of D(−)- and L(+)-isomers [2]. The ratio of poly-L-LA and poly-D-LA modulates the properties and the degradability of PLA, however, D-LA is not suitable for use in the food, drink and pharmaceutical industries because it can cause metabolic problems and is toxic to human body. Thereby, the major challenge lies in producing optically pure LA achieving high concentrations, yields and productivity, using cheap renewable resources [3].

The European paper industry generates huge amounts of waste annually, 70% of which originated from recycled paper production [4]. Thus it is urgent to find cost effective and environmentally sustainable applications of this waste as alternative to its harmful deposition in landfills.

Under a biorefinery approach, the selective production of L(+)-lactic acid from recycled paper sludge (RPS)[3] by simultaneous saccharification and fermentation (SSF)[4] has previously been implemented under a pulsed fed-batch mode using *Lactobacillus rhamnosus* [5]. However, numerous studies have demonstrated that LA promotes an important inhibitor effect both on cell growth and on LA production [6–9]. When a fed-batch strategy is adopted, a high LA concentration is achieved [3], significantly limiting the conversion.

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[1] LA, lactic acid.
[2] PLA, polylactic acid.
[3] RPS, recycled paper sludge.
[4] SSF, simultaneous saccharification and fermentation.

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Different strategies have been proposed to avoid product inhibition on LA fermentation, but product removal of the medium as it is formed is the most effective approach. This can be effectively accomplished through membrane separation processes, with significant advantages in terms of energy efficiency, separation capacity, selectivity and capital investments [10]. A membrane bioreactor, consisting of a membrane separation system coupled to a fermentor, might allow the continuous reuse of enzymes, a crucial feature for the process economics, together with increased capacity and yield by operating at high cell densities and avoiding product inhibition [11]. The objective pursued in this work will be thus the intensification of the SSF process by its implementation into a membrane-recycle bioreactor (MRB),[5] in which the substrate is fed to the fermentor and the reaction mixture is continuously recycled from and to the fermentor through an external filtration unit. It will be possible to maintain a stream with constant product concentration during extended operation by assuring that the product is removed as fast as it is formed [12].

High dry matter contents should be used for running SSF processes so as to obtain reasonable product concentrations. However, the operational feasibility of membrane bioreactors is questionable when handling high-solids loadings, as they deteriorate membrane functionality due to increased cake layer formation and membrane fouling [13]. SSF processes not only deal with suspended growing microbial cells but also with residual lignocellulosic solid material (in this case RPS) providing a very high content of suspended solids. Thereby, according to the authors' knowledge, there are no previously published studies on the use of MRB for implementation of SSF processes. It will be thus very important to adequately select module configuration and process conditions, in manner to decrease the concentration polarization phenomena, with the consequent fouling phenomena. This could be accomplished by using high flow velocities (to increase the mass transfer) and lower transmembrane pressures (TMP).[6] However, this can be insufficient to solve the problem and a dynamic membrane filtration configuration should be adopted to promote higher membrane shear rate.

In the present work, the performance of a purposely built flat sheet filtration module will be assessed by promoting the feed stream pass along the surface at high cross-flow velocity, and thus operating as a dynamic cross-flow filtration system. An ultrafiltration process, with porous asymmetric polymeric membranes, will be implemented. The SSF process deals with cellulases, which might degrade cellulose-based membranes commonly used for micro- and ultrafiltration. As alternative, polysulphone (PS)[7] and polyether sulphone (PES)[8] membranes, exhibiting very good chemical and thermal stability [11], will be utilized.

Given the singularity of the proposed system, the best strategy to run the SSF process for LA production from RPS into the in-house MRB will be investigated based on the product inhibited nature of the process along with the limitations imposed to system operation by the high solids concentrations.

2. Materials and methods

2.1. Materials

Pressed RPS, consisting of the solids collected from the wastewater treatment plant of a local paper recycling mill (Renova, Torres Novas, Portugal), was used as substrate. This sludge was used after neutralization with hydrochloric acid (0.3 g $HCl\,g^{-1}$ oven-dried RPS) and it was milled and sieved into 1.5-mm pieces before use, the same procedure as adopted and justified in a previous work [5]. Each stock was characterized (as described in [14]) to determine exact polysaccharides' content prior to use.

Two commercial enzyme (cellulolytic, also containing highly active xylanases) preparations (from Novozymes, Denmark), Celluclast® 1.5L and Novozym® 188, were purchased from manufacturer.

Lactobacillus rhamnosus ATCC 7469, obtained from the American Type Culture Collection, was used.

2.2. Studies of product inhibition on lactic acid fermentation

To investigate product inhibition, cultivations were firstly carried out at 37 °C, in shake-flasks as described in a previous study [5], using De Man Rogosa and Sharpe (MRS)[9] broth supplemented with reagent-grade glucose at the same concentration of the RPS hydrolysate (56 g L^{-1}) and increasing concentrations of LA (15, 30 and 50 g L^{-1}) with addition of $CaCO_3$ (30, 40 and 50 g L^{-1}, respectively) for buffering effect. To complement this inhibition study, cultivation was carried out under fed-batch mode in a 3-L fermentor (BioFlo II, New Brunswick Scientific, Edison, NJ, USA), equipped with 2 Rushton turbines (with no baffles), with a stirrer speed of 750 rev min^{-1} and no aeration (N_2 was flushed in the beginning and for sampling) so as to assure microaerophilic conditions throughout. The fed-batch experiment was started with 1.3-L initial working volume containing 60 g L^{-1} of glucose. The cultivation proceeded for 10 h in batch, and thereafter a 740-g L^{-1} glucose solution (prepared in MRS broth) was continuously fed to the fermentor. Glucose concentration was frequently monitored by HPLC, with the consequent adjustment of the feeding rate so as to compensate its consumption. pH was maintained at 6.0 by automatic addition of 8 N NaOH.

Most of the cultivations were carried out in replicate, to ensure that consistent profiles are exhibited, and the corresponding results were reported as mean values, with variances below 10%.

2.3. Membrane-based separation process

2.3.1. In-house flat sheet dynamic cross-flow filtration system

A filtration cell, consisting (as displayed in Fig. 1) of an inox cylindrical module where an internal cylindrical acrylic module fits with a circular membrane (71 cm^2), was built for filtration of the SSF suspension. Three different internal acrylic modules (iii–v in Fig. 1b) were built with different inlet and outlet geometries.

The following flat sheet membranes (MICRODYN-NADIR GmbH, Wiesbaden, Germany) were tested: UP010, polyether sulphone (PES) membrane with 10-kDa molecular weight cut-off (MWCO); UP020, PES membrane (20 kDa MWCO); and UH030, permanently hydrophilic polyether sulphone (PESH)[10] membrane (30 kDa MWCO).

2.3.2. Experiments for filtration of the SSF suspension

Filtration experiments with the SSF suspension were carried out at 37 °C (temperature of the SSF process) in a total recycle mode of operation, under different operation conditions: transmembrane pressure (TMP) of 40–100 kPa and high recirculation flow rate (100–225 L h^{-1}) provided by a Watson Marlow (Falmouth, Cornwall, UK) 604 U peristaltic pump.

Suspension obtained in the end (after 96 h) of the batch SSF process, performed in the 3-L fermentor (containing 1.8 L of medium, basically consisting of neutralized RPS supplemented with all the

[5] MRB, membrane-recycle bioreactor.
[6] TMP, transmembrane pressure.
[7] PS, polysulphone.
[8] PES, polyether sulphone.
[9] MRS, De Man Rogosa and Sharpe.
[10] PESH, permanently hydrophilic polyether sulphone.