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Scale-up for esters production from straw whiskers for biofuel applications

Agapi Dima [a], Konstantina Boura [a], Argyro Bekatorou [a], Panagiota-Yiolanda Stergiou [b], Athanasios Foukis [b], Olga A. Gkini [b], Panagiotis Kandylis [a], Katerina Pissaridi [a], Maria Kanellaki [a], Emmanuel M. Papamichael [b], Athanasios A. Koutinas [a,*]

[a] Food Biotechnology Group, Department of Chemistry, University of Patras, 26500 Patras, Greece
[b] Group of Enzyme Biotechnology and Genetic Engineering, Department of Chemistry, University of Ioannina, Ioannina 45110, Greece

HIGHLIGHTS

- Scale-up of anaerobic acidogenesis of wheat straw by a UASB culture.
- The process was promoted by culture immobilization on kissiris.
- Organic acids were produced from straw without a separate hydrolysis step.
- 1-Butanol was the best solvent for acids recovery from the fermentation broth.
- Enzymatic esterification of the acids with 1-butanol resulted to 90% yield.

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ABSTRACT

Delignified wheat straw was fermented by a mixed bacterial anaerobic culture obtained from a UASB reactor to produce organic acids (OAs). Kissiris was used as immobilization carrier in a 2-compartment 82 L bioreactor filled with 17 L of fermentation broth for the first 7 fermentation batches and up to 40 L for the subsequent batches. The amount of straw used was 30 g/L and the temperature was set at 37 °C for all experiments. The total OAs reached concentrations up to 17.53 g/L and the produced ethanol ranged from 0.3 to 1 mL/L. The main OAs produced was acetic acid (6–8 g/L) and butyric acid (3–8 g/L). The OAs were recovered from the fermentation broth by a downstream process using 1-butanol, which was the solvent with the best recovery yields and also served as the esterification alcohol. The enzymatic esterification of OAs resulted to 90% yield.

1. Introduction

Nowadays there is an enormous interest on renewable resources such as waste lignocellulosic biomass for chemicals and biofuels production in order to overcome the ethical and economic issues related to arable land use. Lignocellulosics are the most abundant source of biomass, consisting mainly of cellulose (40–50%), hemicelluloses (25–30%), and lignin (15–20%) (Deng et al., 2014; Menon and Rao, 2012). In this respect, the anaerobic digestion of waste biomass is a promising perspective for energy production. Anaerobic digestion involves four main steps: (1) hydrolytic breakdown of organic compounds into soluble oligomers, (2) acidogenesis and (3) acetogenesis, which include both hydrolysis and fermentation into organic acids (OAs), H_2 and CO_2, and finally (4) methanogenesis to produce CH_4 and CO_2 (Tomei et al., 2009).

In previous studies it was shown that fermentation processes can be promoted in the presence of porous materials such as kissiris and γ-alumina, which acted as culture immobilization carriers and facilitated continuous processing (Galanakis et al., 2012; Lappa et al., 2015; Koutinas et al., 2016). Specifically, the acidogenic fermentation of various sugars and waste resources, promoted by culture immobilization techniques for OAs production has been reported, in some cases with simultaneous production of ethanol (Syngiridis et al., 2014, 2013; Lappa et al., 2015; Koutinas et al., 2016). OAs and ethanol can be used as the reagents of an esterification reaction (enzymatic or chemical) to produce new generation biofuels similar to biodiesel. A limiting factor for such processes is the recovery of the OAs from the fermented broths. A liquid-liquid

* Corresponding author.
E-mail address: a.a.koutinas@upatras.gr (A.A. Koutinas).

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downstream OAs extraction process using various alcohols that could also serve as esterification reagents was previously reported (Bekatorou et al., 2016). Lipase-catalyzed esterification of the recovered OAs from a fermentation broth was subsequently studied, evaluating the effect of process conditions and bioreactor design on the yield and quality of ester synthesis (Stergiou et al., 2013).

Based on the above studies that involved small scale laboratory experiments, the scale-up of anaerobic acidogenesis of wheat straw, promoted by kissiris as immobilization carrier, followed by a scale-up enzymatic esterification of the recovered OAs in a suitable alcohol solvent, were examined in this study.

2. Materials and methods

2.1. Culture and materials

A mixed bacterial anaerobic culture was obtained from a UASB reactor and grown at 37 °C in a medium containing: 50 g/L glucose, NH_3 and 50% H_3PO_4 solution of 100:5:1 COD:N:P ratio, 4 g/L $NaHCO_3$, and 4 g/L yeast extract. The media were sterilized by autoclaving at 120 °C for 15 min. The culture was immobilized on kissiris (Lappa et al., 2015). Delignified wheat straw was used as substrate in all experiments. Delignification took place by boiling 300 g of wheat straw for 3 h in 3 L of 1% w/v NaOH solution (Koutinas et al., 2012). The lignin removal percentage was 62.3% as determined previously (Tsafrakidou et al., 2014). The delignified straw was dried and cut into 1 cm pieces.

2.2. Scale-up acidogenesis of wheat straw

The experimental apparatus consisted of a stainless steel cylindrical tower bioreactor of 82 L total working volume connected with a peristaltic pump in order to achieve effluent recycling (Fig. 1). The bioreactor consisted of two compartments, each made of a metal net basket filled with kissiris. The bioreactor was placed in a incubator set at 37 °C and was also equipped with two sample receivers as well as a vertical transparent cylinder to allow observation of the broth level. An amount of 2.75 kg of kissiris (1 cm particles) was placed in the 1st basket compartment, while 4.4 kg of kissiris (5 cm pieces) were placed in the bioreactor, outside the basket. To start-up the experiment, 2 L of fermented growth medium with 50 g of suspended culture were added, 15 L of fresh liquid were pumped, and 510 g of delignified straw were added. The final composition of the fermenting liquid was delignified straw 30 g/L, 27.2 mL NH_3 and 5.44 mL 50% H_3PO_4, 68 g/L $NaHCO_3$, and 68 g/L yeast extract. Moreover, for immobilization of the culture on kissiris as well as for adaptation in the cellulosic fermentation medium, 200 g of glucose were also added in the bioreactor. The initial pH of the broth was 9. The system was allowed to ferment for 24 h, and then the pump was connected to recycle the fermenting liquid at a rate of 536 mL/min. At the end of each batch (about 10 days duration, when the formed OAs concentration was starting to decline), the liquid was removed, while the leftover straw was maintained in the bioreactor. For the following fermentation batches, additional straw and nutrients were added as above in addition to 2 L of the previously fermented liquid that contained the culture and served as the inoculate. Six batches were performed with this configuration. Subsequently, the 2nd compartment was also loaded with kissiris (2.75 kg; 1 cm particles), and fermentations were performed using only the culture mass that was immobilized on the kissiris of the 1st compartment. Two more fermentation batches were carried out at 40 L total liquid volume. For these fermentations, the liquid medium contained (per litre) 1.6 mL NH_3 and 0.32 mL 50% H_3PO_4, 4 g yeast extract, 4 g $NaHCO_3$ and 30 g delignified straw.

2.3. Recovery of OAs from a fermented broth

The solvents used for OAs recovery were HPLC grade 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol, 2-pentanol, as well as fusel oil (obtained from the alcohol distillery B.G. Spiliopoulos S.A., Patras, Greece). Recovery took place for 30 min at 20 °C under stirring and a solvent/solution ratio of 1:1. The fermented broth used for the study of OAs recovery contained 6 g/L acetic acid, 1.1 g/L propionic acid, 1.1 g/L isobutyric acid, and 8 g/L butyric acid. The recovered OAs were analyzed by HPLC. All experiments were carried out in triplicate.

2.4. Bioreactor and process for scale-up esterification

The esterification of the recovered OAs with 1-butanol was performed using immobilized *Candida antarctica* lipase-B recombinant from *Aspergillus oryzae*. The experimental apparatus consisted of a

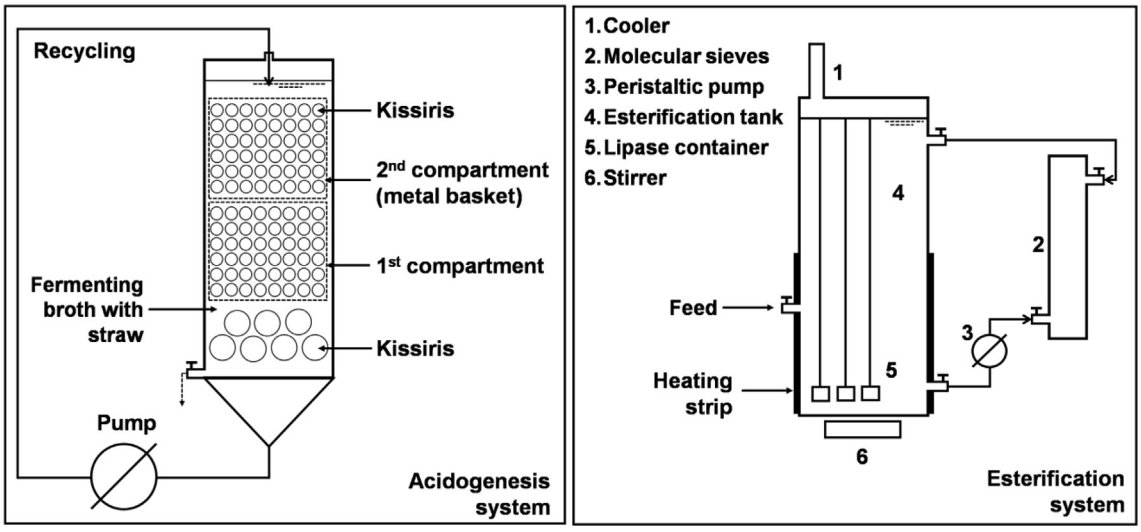

Fig. 1. Bioreactor designs for scale-up acidogenic fermentation of delignified wheat straw using a UASB culture immobilized on kissiris (left), and for enzymatic esterification of the recovered OAs (in 1-butanol) (right).