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Case Study

Butanol production by bioconversion of cheese whey in a continuous packed bed reactor

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HIGHLIGHTS

- Butanol production by bioconversion of renewable resource.
- *Clostridium acetobutylicum* fermentation of unsupplemented cheese whey.
- Operation of a biofilm packed bed reactor.
- Optimization of operating conditions.

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ABSTRACT

Butanol production by *Clostridium acetobutylicum* DSM 792 fermentation was investigated. Unsupplemented cheese whey was adopted as renewable feedstock. The conversion was successfully carried out in a biofilm packed bed reactor (PBR) for more than 3 months.

The PBR was a 4 cm ID, 16 cm high glass tube with a 8 cm bed of 3 mm Tygon rings, as carriers. It was operated at the dilution rate between 0.4 h^{-1} and 0.94 h^{-1}.

The cheese whey conversion process was characterized in terms of metabolites production (butanol included), lactose conversion and biofilm mass. Under optimized conditions, the performances were: butanol productivity 2.66 g/Lh, butanol concentration 4.93 g/L, butanol yield 0.26 g/g, butanol selectivity of the overall solvents production 82 wt%.

1. Introduction

The socio-economic scenario of the beginning of the third millennium revives the interest in a strategy for the bioconversion of industrial wastewaters in biofuels and bulk chemicals. In this context, acetone–butanol–ethanol (ABE) fermentation is being considered as a way to upgrade renewable resources into valuable base chemicals and liquid fuels (Ezeji et al., 2007a; Dürre, 2007; Friedl, 2012). Indeed, butanol offers several advantages over ethanol for gasoline–alcohol blending because of its high energy content, low miscibility with water, and low volatility (Bohlmann, 2007; Cascone, 2008). In addition, butanol can replace gasoline with no need to modify the current vehicle and engine technologies.

ABE is typically produced during the last stage of batch fermentation of some *Clostridium* strains – saccharolytic butyric acid-producing bacteria – under appropriate operating conditions (*Clostridium saccharoperbutylacetonicum*, *C. acetobutylicum*, *C. beijerinckii*, *C. aurantibutyricum*). The strains are able to metabolize a great deal of substrates: pentoses, hexoses, mono-, di- and polysaccharides (Flickinger and Drew, 1999). Under batch conditions, the fermentation process of solvent-producing clostridia proceeds with the production of cells, hydrogen, carbon dioxide, acetic acid and butyric acid during the initial growth phase (acidogenesis) (Jones and Woods, 1986). As acid concentration increases (pH decreases), the cell metabolism shifts to solvent production (solventogenesis) and acidogenic cells – able to reproduce themselves – shift to the solventogenesis state with a morphological change (Jones and Woods, 1986). Under solventogenesis, the active cells become endospores unable to reproduce themselves. Accordingly, two different physiological states must be taken into account for clostridia: one for the acidogenic phase, and one for the solventogenic phase.

Despite the aforesaid remarkable advantages of butanol as a bioproduct, its industrial production via fermentation is not developed for several issues: feedstock cost and availability, low yield and productivity, low concentration of butanol in the broth (as a consequence of the product-inhibition feature of ABE fermentation), degeneration of butanol-producing strains (Kumar and Gayen, 2011). Moreover, the low butanol concentration in fermentation broth makes its recovery and concentration quite complex (Liu and Fan, 2004; Ezeji et al., 2007b, 2013; Napoli et al., 2012a).

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Reactor performance enhancements were proved by continuous ABE production in reactors operating with *clostridium* cell-confinement options: cell-immobilisation (Ezeji et al., 2007b; Lee et al., 2008; Qureshi et al., 2000; Napoli et al., 2010; Lu et al., 2012) or cell-recycling (Meyer and Papoutsakis, 1989; Tashiro et al., 2005; Zheng et al., 2013). As regards cell-immobilized reactors, their high cell density improves butanol yield and butanol recovery. Continuous bioconversion in immobilized cell reactors has several advantages over batch cultures, which are typically adopted for butanol production via fermentation (Qureshi et al., 2000). The main advantages are related to the high cell concentrations and to the reactor operability at high dilution rates without cell washout (Welsh et al., 1987). Moreover, the cell support may often be reused (Krouwel et al., 1980).

As regards the feedstock, abundance and un-competitiveness with food sources are prerequisites of potential substrates. Generally, lignocellulosic biomass and wastewater streams have these requisites. The former may be cultivated ad hoc or obtained as agro-industrial by-products. The latter include cheese-whey and wastewaters. The potential of cheese-whey as feedstock for butanol production has been pointed out by several authors (Qureshi and Maddox, 1987; Gonzalez-Siso, 1996; Foda and Dong, 2010). The tests have always focused on batch fermentations (Foda and Dong, 2010) and continuous bioreactors fed with whey permeate supplemented with yeast extract (Qureshi and Maddox, 1987).

The aim of this contribution is to investigate the feasibility of bio-butanol production by continuous conversion of cheese whey, a dairy industry wastewater characterized by high lactose and protein content. The anaerobic solventogenic bacterium *C. acetobutylicum* DSM 792 was adopted for the fermentation processes. The conversion was carried out in a packed bed reactor (PBR) of Tygon rings as biofilm carriers. The process was characterized in terms of butanol production rate, butanol selectivity and butanol yield as a function of the dilution rate.

2. Methods

2.1. Microorganism

C. acetobutylicum DSM 792 was supplied by DSMZ. Stock cultures were reactivated according to the DSMZ procedure. Reactivated cultures were stored at −80 °C. The thawed cells were inoculated in 15 mL Hungate tubes containing 12 mL of synthetic medium: 20 g/L lactose, 5 g/L yeast extract (YE). Cells were grown under anaerobic conditions for 48 h at 37 °C. Then pre-cultures were transferred into fermentation bottles.

2.2. Medium

2.2.1. Synthetic medium

The synthetic medium consisted of 20 g/L lactose and 5 g/L YE supplemented with P2 stock solution: buffer – 0.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4; 2 g/L ammonium chloride; mineral – 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $MnSO_4{\cdot}H_2O$, 0.01 g/L $FeSO_4{\cdot}7H_2O$ (Qureshi and Blaschek, 2000). The lactose–YE solution was sterilized in autoclave (20 min at 120 °C).

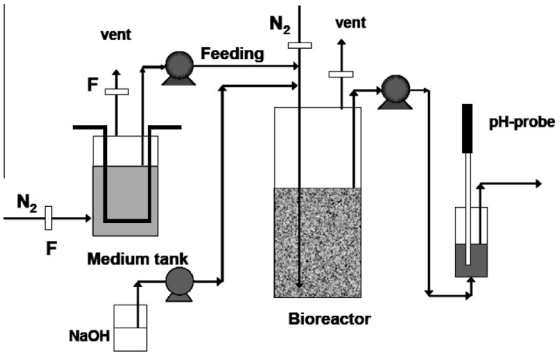

Fig. 1. Outline of the apparatus adopted for continuous test equipped with a packed bed biofilm reactor. F – gas sterilization filter.

2.2.2. Cheese whey powder

The cheese whey powder (CWP) was by Sierolat, an Italian company. The composition of the powder was: lactose 69 wt%, proteins 12.5 wt%, ashes 7.5 wt%, lactic acid 4 wt%, moisture 2.5 wt%, fats 2.5 wt%, galactose 2 wt%.

The CWP was solubilized in deionized water (concentration 40 g/L) and pretreated according to the methods reported in Table 1.

The continuous biofilm reactor was fed with a pretreated solution of CWP characterized by 28 g/L lactose concentration.

2.3. Apparatus and operating conditions

All the experiments were carried out at 37 °C.

2.3.1. Batch fermentation

Pyrex screw capped bottles (100 mL) containing 75 mL medium were used as fermenters. Tests were carried out in fermenters at rest without pH control. The medium was inoculated with 6.25% (v/v) suspension of active growing pre-cultures. 3 mL of cultures were periodically sampled to measure the concentration of lactose and metabolites.

2.3.2. Continuous bioreactor

The apparatus adopted for the cheese whey fermentation consisted of a fixed bed reactor, liquid pumps, a heating apparatus, a device for pH control, and on-line diagnostics (Fig. 1). The fixed bed was at the bottom of a 200 mL glass lined pipe (4 cm ID, 16 cm high) jacketed for the heat exchange. The liquid phase volume in the reactor, or reaction volume, was modified by changing the level of the overflow duct. Nitrogen was sparged at the reactor bottom to support anaerobic conditions. The device for pH control consisted of a pH-meter, a peristaltic pump, a vessel with NaOH 0.3 M solution, and a controller. The reactor with the carriers was sterilized in autoclave at 121 °C for 20 min. The gas stream was

Table 1
Cheese whey pretreatment methods. Main results.

Pretreatment method		Max butanol [g/L]	Residual acids [g/L]	Clot presence
Heat sterilization	inoculum	6.4	2.7	Yes
	Control	0	0	
Tyndalization	inoculum	6.3	2.7	Yes
	Control	0	0.1	
Dry tyndalization	inoculum	8.1	2.7	No
	Control	0	6.8	
Deproteinization	inoculum	8.9	2.3	No
	Control	0	0	