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Butanol production by *Clostridium acetobutylicum* in a series of packed bed biofilm reactors

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HIGHLIGHTS

- Butanol production was performed by immobilized cells of *C. acetobutylicum*.
- The process was performed in 4 biofilm packed bed reactors connected in series.
- The biofilm was assumed to be formed of an active shell and an inner inactive layer.

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ABSTRACT

The continuous production of Acetone, n-Butanol and Ethanol (ABE) by immobilized cells of *Clostridium acetobutylicum* DSM 792 using glucose and lactose as carbon source is presented in this paper. The conversion process was successfully carried out for more than three months in 4 packed bed biofilm reactors (PBBRs) connected in series. The first PBBR of the series (fed with fresh medium) was kept under acidogenesis conditions and the three other PBBRs were kept under solventogenesis conditions.

Each PBBR was a glass tube (4 cm ID, 8 cm high) with a 4 cm-bed of 3 mm-Tygon rings as carriers. The PBBR system was fed with 100 g/L of lactose medium. The fermentation process was characterized in terms of metabolite production (butyric and acetic acids, acetone, butanol, and ethanol), sugar conversion and mass of biofilm. The overall dilution rate (D_{TOT}) was varied between 0.15 h^{-1} and 0.9 h^{-1} to assess the PBBR system performance as a function of D_{TOT}. The best PBBR system performance under optimized conditions was: butanol productivity 9.2 g/Lh, butanol concentration 10.8 g/L, acetone concentration 2.4 g/L, ethanol concentration 1.8 g/L, selectivity of butanol with respect to all solvents 72%$_w$. To the authors' knowledge, these butanol productivity and concentration values are the highest in the literature on lactose/(cheese whey) fermentation.

An interpretation of the biofilm structure in the PBBR was put forward.

1. Introduction

The development of biotechnological processes to produce butanol – a second-generation biofuel and a chemical building block – from renewable sources to find eco-sustainable alternatives to the petrochemical routes (Kumar and Gayen, 2011) is still an open challenge. ABE fermentation by clostridia is drawing new interest as a way to turn renewable resources into valuable base chemicals and liquid fuels (Sarchami and Rehmann, 2014; Dürre, 2007; Friedl, 2012). Butanol offers several advantages over ethanol for gasoline-alcohol blending: 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 (Cascone, 2008).

Nevertheless, its low yield, the consequent acid-solvent production and low concentration of butanol due to its inhibiting effect on fermentation have hindered its success on an industrial scale so far. The most preferred substrates used in the traditional batch fermentation – starch and molasses – result in a total yield of 30–32% and butanol concentration of about 10–15 g/L (Kumar et al., 2012).

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Nomenclature

AA	Acetic Acid
AA^{UP}	Concentration of the converted Acetic Acid
ABE	Acetone-Butanol-Ethanol
BA	Butyric Acid
BA^{UP}	concentration of the converted Butyric Acid
CSTR	Continuous stirred tank reactor
D	Overall Dilution rate
YE	Yeast Extract
PBBR	Packed Bed Biofilm Reactor
PFR	Plug flow reactor
r_B	Specific butanol production rate
S	Sugar concentration
W_{ABE}	ABE productivity
W_B	Butanol productivity
W^{UP}_{Acids}	Rate for volume unit of converted acids
W^{Net}_{Acids}	Rate for volume unit of produced acids
W^{TOT}_{Acids}	Total acids production rate
$X_{A,active}$	Acidogenic active cells concentration in the biofilm layer
$X_{S,active}$	Solventogenic active cells concentration in the biofilm layer
$X_{inactive}$	Inactive cells concentration in the biofilm layer
X_{TOT}	Total cell concentration
$Y_{i/L}$	Sugar-to-"i-species" fractional yield coefficient
ξ_S	Sugar conversion degree
Φ	Butanol to ABE selectivity

Reactor design and operating conditions play a key role in fermentative productions (Schugerl, 1997). The main factors that hinder the commercial development of the traditional batch fermentation processes include low cell density, low reactor productivity, high down-times, nutritional limitations and severe product inhibition (Chen and Blaschek, 1999). Improved performance can be obtained by increasing cell concentration in cell immobilized reactors and retention membrane reactors (Qureshi et al., 2005). Reactor performance can also be enhanced with continuous ABE production in reactors operated with *clostridium* cell-confinement options: cell immobilisation (Gapes et al., 1996; Yen et al., 2011; Viikiläät et al., 2013; Lee et al., 2008; Qureshi et al., 2000) or cell recycling (Meyer and Papoutsakis, 1989; Tashiro et al., 2005; Procentese et al., 2015a, 2015b). In cell immobilization biofilm reactors, their high cell density allows for better butanol yield and recovery. Continuous bioconversion presents several advantages over batch cultures in biofilm reactors (Qureshi et al., 2000). The main advantages are related to the high cell concentration and to the reactor operating at high dilution rates without cell washout (Welsh et al., 1987). Moreover, the biofilm support can be reused (Krouwel et al., 1980).

Fig. 1 is the map of butanol productivity and concentration in the final product proposed by Setlhaku et al. (2013) for continuous bioreactor systems, as reported in the open literature.

Butanol productivity and concentration are benchmarked against $W_{B,min} = 0.24$ g/Lh and $B_{min} = 13$ g/L, which are reported as the best batch fermentations with clostridia in the literature (Jones and Woods, 1986). A butanol productivity value of 5 g/ Lh ($W_{B,industry,min}$) is suggested as the industrial hurdle rate. The figure also indicates the minimum butanol target ($B_{DSP,min}$) of 36 g/L calculated as the threshold for successful butanol recovery by an energy optimized distillation unit (Mariano and Filho, 2012). To the authors' knowledge and as confirmed in Fig. 1, with the biofilm reactors described in the literature the final concentration of solvents obtained is too low to get an efficient butanol recovery, in particular by a distillation unit. Such a low butanol concentration is likely to depend on the impossibility to keep the two phases of the clostridia fermentation (acidogenesis and solventogenesis) separate when a single-stage reactor is used.

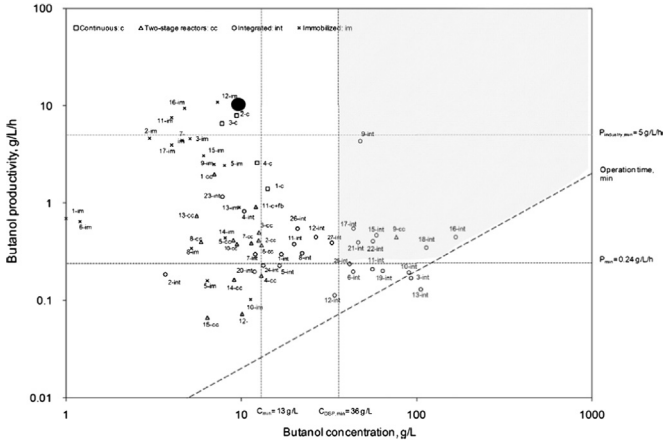

Fig. 1. Window of operation for ABE continuous, two-stage reactors, immobilized and product integrated fermentations (Setlhaku et al., 2013). The full circle represents the best performance obtained in the test herein.

The study reported in this paper is about continuous bio-butanol production by lactose conversion in an innovative immobilized cell reactor system. Lactose was chosen as carbon source because it is the main component of cheese whey, a very common by-product of the dairy industry (Raganati et al., 2013). The anaerobic solventogenic bacterium *Clostridium acetobutylicum* DSM 792 was used for the fermentation process. The conversion was carried out in 4 packed bed biofilm reactors (PBBRs) connected in series: the first reactor (fed with the carbon source) was kept under acidogenesis conditions, and the three other reactors were kept under solventogenesis conditions. This series configuration allowed to keep the two phases of the ABE fermentation separate: acids were produced in a section of the system and then the acids and the residual sugar were converted in solvents in the following section. The PBBR system performance was characterized in terms of final butanol concentration and productivity and measured as a function of the dilution rate. A possible interpretation of the biofilm structure, based on the non-homogenous nature of the substrate/metabolite concentration across the biofilm is also put forward.

2. Materials, methods and procedures

2.1. Microorganism

Clostridium acetobutylicum DSM 792 was supplied by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). Stock cultures were reactivated according to the procedure indicated by the supplier. The reactivated cultures were stored at −80°C. After thawing, the reactivated cultures were inoculated in 15 mL Hungate tubes containing 12 mL synthetic medium (see the following paragraph) supplemented with 30 g/L lactose and 5 g/L yeast extract (YE). The cells were grown under anaerobic conditions for 48 h at 37 °C.