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Continuous lactose fermentation by *Clostridium acetobutylicum* – Assessment of solventogenic kinetics

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HIGHLIGHTS

- Butanol production by ABE fermentation in a CSTR-microfiltration unit is reported.
- Lactose was adopted as carbon source to mime cheese-whey.
- Solventogenesis of ABE fermentation was characterized under steady state conditions.
- A product inhibition model was adopted for the butanol production rate.

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ABSTRACT

This work reports the results of a series of tests on the specific butanol production rate by *Clostridium acetobutylicum* continuous cultures. The tests were carried out using lactose as carbon source to mimic cheese-whey. A continuous stirred tank reactor equipped with a microfiltration unit was used. The dilution rate (D) ranged between 0.02 and 0.15 h^{-1} and the ratio R of the permeate stream rate to the stream fed to the reactor ranged between 14% and 95%. For each set of D and R values, the continuous cultures were characterized in terms of concentration of cells, acids and solvents. Results were processed to assess the concentration of acidogenic cells, solventogenic cells, spores and the specific butanol production rate. The max butanol productivity was 0.5 g L^{-1} h^{-1} at $D = 0.1$ h^{-1} and $R = 95\%$. The butanol productivity referred to solventogenic cells was expressed as a function of concentration of lactose, acids and butanol.

1. Introduction

Despite the awareness of the environmental damages caused by fossil-derived products/processes, the worldwide demand of fuels as well as of chemical building blocks is continuously increasing while the availability of fossil fuel sources is diminishing. A potential solution is the development of alternative routes to the petrochemical productions. Butanol is an alternative fuel that can be produced by alternative thermochemical and biotechnological routes. Butanol can also be used (Cascone, 2008; Lee et al., 2008; Jin et al., 2011): as a solvent (e.g., for paints, coatings, varnishes, resins, gums, dyes); in cosmetics (e.g., nail care products, eye makeup, lipsticks, shaving products, personal hygiene products); as a building block of chemicals (e.g., butyl acrylate, methacrylate). The main differences with respect to ethanol – a well-known biofuel – are (Durre, 2007; Lee et al., 2008): the Lower Heating Value is 25% larger; it is 6 times less evaporative; it is less hygroscopic; it may be transported in the existing gasoline systems. Butanol is 13.5 times less evaporative than gasoline and can be mixed with it at high ratios to fuel existing cars with no need to modify gasoline-based engines. Several companies claim that butanol can be used as a total replacement fuel for gasoline without any modifications to car engines. Indeed, several companies have already taken the butanol bet, like Sovert, ButalcoGmBH in Europe, Environmental Energy Inc., GEVO™, Butamax™, Cobalt Technologies) in the U.S. and Cathay Industrial Biotech in Asia.

Acetone–butanol–ethanol (ABE) fermentation is one of the biotechnological routes to the production of butanol. It was proposed by Weizmann and Rosenfeld (1937) and was widely applied during the first half of the 20th century. With the advent of the petrochemical industry in the second half of the last century the production of acetone and butanol by fermentation became uncompetitive and the plants were shut down. Nowadays, the

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Nomenclature

AA, Ac, B, BA, Et concentration of acetic acid, acetone, butanol, butyric acid, ethanol ($g\ L^{-1}$)
AA_{max}, BA_{max}, Ac_{max}, B_{max}, Et_{max} critical concentrations of acids and solvents ($g\ L^{-1}$)
D_i dilution rate with respect to the stream "*i*" (h^{-1})
F_i molar net rate of production/uptake of the species "*i*" assessed experimentally
f_n^i molar rate of the production/uptake of the species "*i*" referred to the metabolic reaction *n*
K_L, $K_{L,B}$ constant ($g\ L^{-1}$)
K_B inhibition constant ($g\ L^{-1}$)
C_L lactose ($g\ L^{-1}$)
μ specific growth rate (h^{-1})
n_{AA}, n_{BA}, n_{Ac}, n_{Et} and n_B parameter of Eq. (14)
R recycle ratio
ξ_L lactose conversion degree
$Y_{i/L}$ lactose-to-"*i*-species" fractional yield coefficient
X, X_A, X_S, X_D concentration of total cells, acidogenic cells, solventogenic cells and spores ($g_{DM}\ L^{-1}$)
W_X, W_X^A, W_X^S, W_X^D mass flow rate of total cells, acidogenic cells, solventogenic cells and spores ($g_{DM}\ L^{-1}\ h^{-1}$)

progress of research has revived the interest in the fermentative route to butanol production (Ezeji et al., 2007; Raganati et al., 2013; Pinto Mariano et al., 2013) but there are still many limitations to its industrial application: the high cost of the substrate; the low product concentrat ion and productivity in fermentation due to end-product inhibition (Jones and Woods, 1986; Zeng et al., 1994); the high product recovery cost (Ezeji et al., 2005; Napoli et al., 2012a). Using a cheap substrate for the fermentation process is part of the solution, the substrate cost accounting for about 60% of the overall production cost. But the availability of a feedstock with a high mass rate during the year and at low cost remains crucial to make butanol fermentation competitive.

Cheese-whey is a wastewater released from the cheese industry. It is produced all over the year at an almost constant rate and at a rather high mass rate. A small dairy produces more than 20 $m^3\ day^{-1}$ of wastewater, the equivalent of a community of about 10,000 people. Its Biochemical Oxygen Demand (BOD) is very high – typically more than 2000 $mg\ L^{-1}$ – and its COD is about 50–70 $g\ L^{-1}$ (Najafpour et al., 2008). Lactose, usually present at a concentration over 30–50 $g\ L^{-1}$, is the main responsible for the polluting behavior of cheese-whey. As a highly polluted stream, cheese-whey must be remediated before being delivered to a sewer system. The scenario is even worse in Italy where there are about 2800 dairies that produce between 8 and 10 million $m^3\ year^{-1}$ of cheese-whey.

ABE is typically produced during the last stage of the batch fermentation of some *Clostridium* strains (*Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium aurantibutyricum*). The *C. acetobutylicum* batch fermentation is characterized by two phases: an acidogenic and a solventogenic phase. During the acidogenic phase the cells grow and produce: acids (butyric and acetic acid), carbon dioxide, and hydrogen. The production of acids causes a decrease in pH, the *C. acetobutylicum* cells adapt themselves to the acid environment by a metabolic and a morphological shift. Then the solventogenesis phase starts: it is characterized by (i) the exponential growth phase coming to an end; (ii) the active cells becoming endospores, unable to grow; (iii) the acids being converted to solvents, acetone–butanol–ethanol (typical molar ratio 3:6:1). The fermentation process ends when high inhibiting solvent concentration (20–30 $g\ L^{-1}$) is reached: the harsh conditions induce cell sporulation and/or cell lyses (Jones and Woods, 1986; Tracy et al., 2008).

During the solventogenesis phase the cell growth rate is quite low. Therefore, a continuous process reactor configuration must be chosen to prevent the reactor washout (Huang et al., 2004; Napoli et al., 2010; Raganati et al., 2013). To properly select and optimize the continuous reactor it is necessary to know the kinetics – cell growth rate, butanol production, etc. – of the process as a function of the operating conditions and of the species involved in the fermentation. The kinetics under solventogenic conditions plays a key role in optimizing the butanol production. Meyer and Papoutsakis (1989a,b) reported a study on the solventogenesis phase with a continuous stirred tank reactor equipped with a microfiltration unit to confine the solventogenic cells in the reactor. They investigated the metabolite production at different dilution rates (D = volumetric flow rate/reaction volume) and different recycle ratios (R – ratio between the volumetric flow rate permeated across the microfiltration unit and the feed flow rate). These authors focused only on solventogenic cells even though a population of acidogenic cells is also found in a continuous reactor (Zheng et al., 2013; Napoli et al., 2011, 2012b).

This work reports the characterization of the *C. acetobutylicum* kinetics under solventogenic conditions. This contribution integrates the model proposed by Napoli et al. (2009) for acidogenic cells. In a future research work the acidogenic cell model and the solventogenic cell model will be combined to support the design of continuous reactors. A continuous stirred tank reactor equipped with a microfiltration unit was used to confine the solventogenic cells in the reactor. The dilution rate and the recycle ratio (the ratio between the volumetric flow rate through the microfiltration unit and the feed flow rate) ranged over wide intervals. Lactose was fed as carbon source to mimic cheese-whey. The fermentation tests were aimed at assessing: (i) the butanol production rate as a function of the operating conditions; (ii) the kinetics during the solventogenic phases.

2. Methods

2.1. Microorganism and media

C. acetobutylicum DSM 792 was supplied by DSMZ. The stock cultures were reactivated according to the method suggested by the supplier and stored at −80 °C. The cells were inoculated into 12 mL of synthetic pre-culture medium containing glucose (30 $g\ L^{-1}$) as carbon source. The carbon-less medium consisted of: 5 $g\ L^{-1}$ of yeast extract, 2 $g\ L^{-1}$ of ammonium chloride (nitrogen source), 0.5 $g\ L^{-1}$ KH_2PO_4 – 0.5 $g\ L^{-1}$ of K_2HPO_4 (buffer), 0.2 $g\ L^{-1}$ of $MgSO_4{\cdot}7H_2O$, 0.01 $g\ L^{-1}$ of $MnSO_4{\cdot}7H_2O$, 0.01 $g\ L^{-1}$ of $FeSO_4{\cdot}7H_2O$ (minerals). The medium was sterilized in autoclave (121 °C, 20 min). The carbon source used during the continuous tests was lactose at 50 $g\ L^{-1}$. The chemicals and the yeast extract were from Sigma Aldrich.

2.2. Apparatus

The continuous fermentation tests were carried out in the apparatus sketched in Fig. 1 consisting of a fermenter, a pH controller