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Production Of Butanol In A Continuous Packed Bed Reactor Of *Clostridium Acetobutylicum*

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The present contribute reports on butanol production by means of biofilm of *Clostridium acetobutylicum* ATCC 824A. The establishment of a stable biofilm and the progress of Acetone-Butanol-Ethanol (ABE) production by immobilized cells are demonstrated in a packed bed reactor using hydrophobic plastic carriers. The medium was a solution of lactose and yeast extract to emulate the cheese whey, an abundant lactose-rich wastewater.
The reactor was operated under controlled conditions with respect to pH and dilution rate. The pH values ranged between 4 and 6, the dilution rate between 0.54 h^{-1} and 2.4 h^{-1} (2.5 time the maximum specific growth rate assessed for suspended cells). The conversion process was characterized in terms of concentration of suspended cells and metabolites and of mass of biofilm. Results were worked out to assess the rate of ABE production.

1. Introduction

Butanol stems out as an attractive substitute "green" biofuel and chemical feedstock (Cascone, 2008). The biotechnological route to butanol production is gaining increasing consideration as the progress of research discloses the many hidden features of the reaction pathways and provides better knowledge-based approaches to process setup and optimization.
The Acetone-Butanol-Ethanol (ABE) fermentation route – despite widely adopted during the first half of the last century– is still open to investigation. Though *clostridia* have been shown to be effective biocatalysts for ABE fermentation, there is still a lack of information in the literature supporting scale up and demonstration at the industrial scale (Jones and Woods, 1986; Cascone, 2008). Only a few attempts are reported in the literature regarding continuous fermentation by means of *clostridia* strains confined in the reactor by immobilization or cell-recycling.
The proven ability of these strains to utilize a wide spectrum of carbohydrates (Flickinger and Drew, 1999) has stimulated research on the use of cheap renewable feedstocks. Along this line, the large carbohydrates content of some wastewater streams,

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e.g. effluents form dairy industries, makes such wastewaters potential low-cost feedstocks for butanol production

Cheese whey has attracted interest as an alternative substrate for ABE fermentation.

Previous studies addressing AB fermentation using whey or lactose as carbon source (Welsh and Veliky, 1984; Ennis and Maddox, 1985; Linden *et al.*, 1986) have pointed out that these substrates are characterized by rather low overall reactor productivities when batch conversions are considered, of the order of 0.1 g/Lh. On other hand, the selectivity to butanol is larger than that typically recorded during the fermentation of conventional substrates, resulting in more favorable economics of butanol recovery.

The present study reports on butanol production by means of *Clostridium acetobutylicum* ATCC 824A. Previous studies addressed conversion of lactose by free cell fermentation under either batch or continuous conditions (Napoli et al., 2009). The present study moves one further step toward the characterization of butanol production by *C. acetobutylicum*. In particular, the study is focused on the development of a biofilm reactor. A packed bed typology (PBR) has been investigated as an intermediate step towards the development of a fluidized bed biofilm reactor. A solution of lactose and yeast extract has been adopted as medium to mime cheese whey wastewater. The adopted medium allowed tuning operating conditions without the typical properties fluctuations of the wastewaters. The butanol production process was characterizes in terms of lactose and metabolites concentrations, solvents productivity, butanol selectivity and lactose conversion rate.

2. Materials And Procedure

2.1 Microorganism, media and support

Clostridium acetobutylicum DSM 792 was supplied by DSMZ. Reactivation and storing procedures as well as stock medium are reported in Napoli et al. (2009).

The culture medium adopted consisted of Yeast Extract (YE) at 3 g/L and of D-Lactose at concentration ranging between 15 and 30 g/L. The medium was sterilized in autoclave.

Tygon® rings were adopted as packed bed solids. Rings had length 0.5 cm, ID 3.2mm and 1.5mm thickness.

2.2 Apparatus

Figure 1 shows a sketch of the apparatus adopted for the lactose fermentation. It consists of a fixed bed reactor, liquid pumps, a heating apparatus, a device for the pH control and on-line diagnostics.

The glass lined and jacketed bioreactor (250 mL volume) was equipped with ports for injecting gas streams, inoculation and sampling. The device for the pH control consisted of a pH-meter, a peristaltic pump, a vessel with NaOH 1M solution and a pH-controller. The anaerobic conditions were achieved and preserved sparging sterile oxygen-free nitrogen at the reactor bottom.

The sterile medium was fed at the bottom of reactor by means of a peristaltic pump. The reactor, with medium and the biofilm supports (Tygon® rings), was sterilized in autoclave. The gas stream was sterilized by filtration. The medium in the stainless steel tanks was also sterilized.

2.3 Diagnostics

Analysis of culture samples withdrawn from the fermenter was carried out after centrifugation at 11,000 rpm for 10 min. The free cells were characterized in terms of biomass concentration. The liquid phase was characterized to measure lactose and metabolites concentration. Lactose concentration (L) was measured by means of an enzymatic kit (Biopharm®). Metabolites concentrations were measured by means of a gas-chromatograph, equipped with a FID and with a capillary column poraplot Q (25 m x 0.32mm), adopting external standards. In particular, the concentration of acetic acid (AA), butyric acid (AB), acetone (Ac), Ethanol (Et) and butanol (B) were measured.

2.4 Operating conditions and procedure

1 mL of stock culture was transferred in a 15 mL screw-cap bottles containing 50 mL of culture media (15 g/L of lactose). The culture was incubated for 2 days under batchwise anaerobic sterile conditions, then 10 mL of active culture was inoculated in the packed bed reactor (PBR).

Tests were carried out with the biofilm PBR operated at selected conditions. Each steady state was characterized in terms of metabolites and lactose concentration. The mass of biofilm in the reactor was assessed at the end of the run.

All tests were carried out at 35°C. The pH set-point was investigated in the range between 4.0 and 5.0.

3. Results

Figure 2 reports data measured during the start up of the reactor loaded with 69 g of Tygon® rings. The concentration of metabolites and the pH are reported as a function of the time. The reactor was inoculated at t=0 and operated batchwise with respect to the liquid phase for 20 hours, a time interval sufficient for the biofilm formation

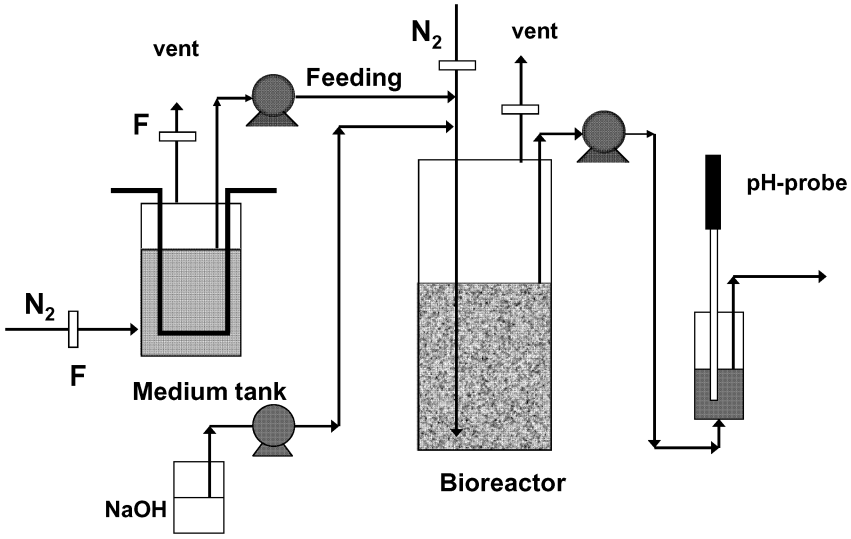

Figure 1 Outline of the apparatus adopted for continuous tests equipped with a packed bed biofilm reactor. F) gas sterilization filter.

(Qureshi *et al.,* 2005). Thereafter, the reactor was switched to continuous operation by steadily feeding the lactose 15 g/L medium. The dilution rate was set at D=0.40 h^{-1} and the pH was gradually increased from 5.0 to 5.5 to force the fermentation under acidogenesis conditions, then under biofilm/cell growth. After about two days of incubation the carriers were covered by a light layer of biofilm visible to the direct observation and the dilution rate was increased to promote biofilm production with respect free cell growth. At D=0.80 h^{-1} - a value close to the maximum specific growth rate of free cells under the operating conditions adopted (Napoli et al., 2009) – the lactose concentration in the reactor decreased at a rate lager than the parallel increase of metabolites concentration, probably as the consequence of the biofilm growth.
The dilution rate was still increased at t=140 h to compensate for the gradual lactose depletion (L<2 g/L). In particular, D was set at 2.4 h^{-1}, about 2.5 times the maximum specific growth rate (Napoli et al., 2009). Under the selected conditions the biofilm reactor approached a steady state regime since t=190 h. All together, the biofilm reactor

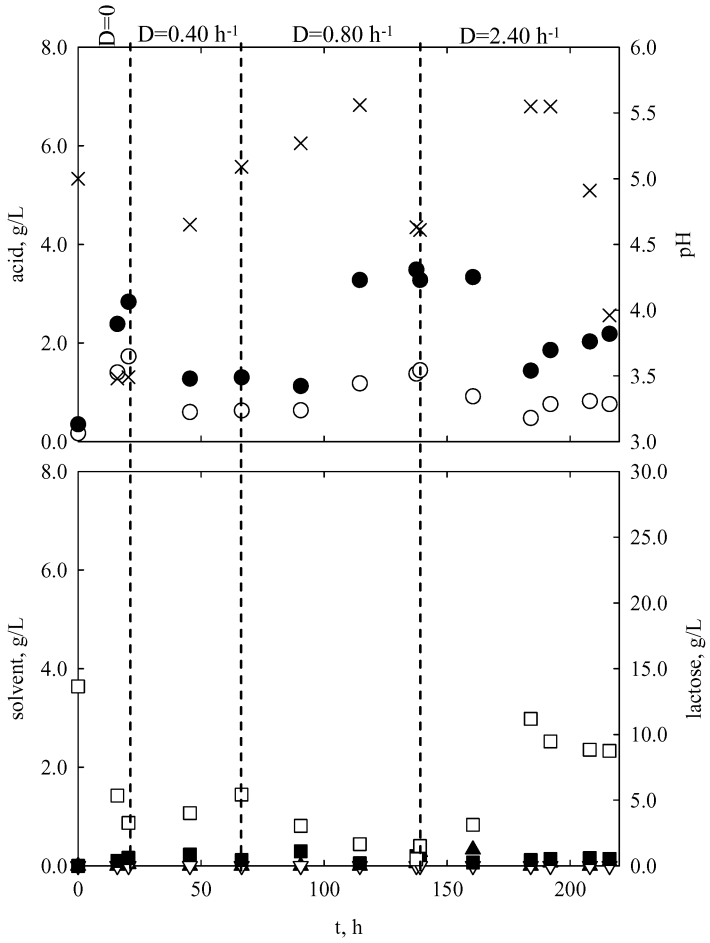

Figure 2 Variables recorded during PBR start up. Lactose concentration in the feeding: 15 g/L. × *pH,* ● *acetic acid,* ○ *butyric acid,* □ *lactose,* ■ *butanol,* ▽ *acetone,* ▲ *ethanol.*

start up took about 9 days and a remarkable amount of biofilm formed.
Provided a substantial amount of biofilm, at t=216 h the bioreactor operating conditions were set to produce butanol: pH at 4.0 and the D at 0.54 h^{-1}. The value of pH was set in agreement with the previous investigation carried out in batch reactor (Napoli et al., 2009): cells shift to the solventogenesis phase at pH=4.0. Figure 3 reports the concentration of lactose and metabolites measured in the reactor for the continuous culture carried out at D=0.54 h^{-1}. As expected the solvents were continuously produced, besides the acids, confirming the co-existence of *C. acetobutylicum* cells voted to produce butanol and acetone and of cells committed to produce biomass. However, lactose conversion and solvents started gradually to decrease along the time highlighting a progressive extinction of the fermentation process. The lactose conversion and solvent production was prompted recovered as pH was increased pH up to 4.3 at t=287 h. Steady state conditions were approached in about two days and lasted for about 6 days (about 60 time-space).
Table 2 reports selected data regarding the steady state characterized by D=0.54 h^{-1} and

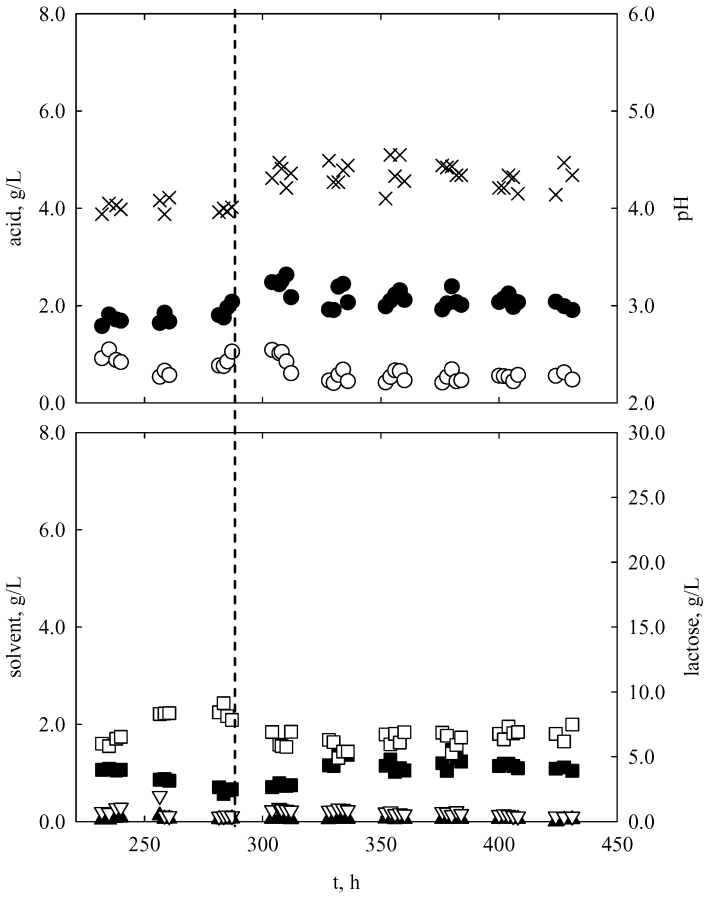

Figure 3 Variables recorded during steady operation of the PBR: D=0.54 h^{-1}; lactose concentration in the feeding: 15 g/L. Key to symbols in fig. 3. The vertical line marks the pH increase.

Table 1 *Steady state cultures of C. acetobutylicum in biofilm packed bed reactor.*

Operating conditions		
	D [h^{-1}]	0.54
	pH	4.34
	Lactose in the feed [g/L]	15.0
Results		
	Metabolites [g/L]	
	Ethanol	0.05
	Acetone	0.18
	Butanol	1.20
	Acetic acid	0.53
	Butyric acid	2.12
	Lactose [g/L]	6.3
	Solvent Productivity [g/Lh]	
	Ethanol	0.03
	Acetone	0.10
	Butanol	0.65

pH=4.3. The reactor performance was characterized in terms of lactose and metabolites concentration, lactose conversion degree, acids and solvents yield, solvents productivity and butanol to solvent selectivity. Notwithstanding the operating conditions adopted promoted the solvents production, the acids production (1.43 g/Lh) was still remarkable with respect to the solvent production (0.77 g/Lh).
Increasing the dilution rate and the pH the reactor performance improved.

4. References

Cascone, R., 2008, Biobutanol - A Replacement for Bioethanol?. Chem Eng Prog, August, S4-S9

Ennis, B.M. and Maddox I.S., 1985, Use of *Clostridium acetobutylicum* P262 for production of solvents from whey permeate, Biotechnol. Lett. 7, 601-606.

Flickinger, M. C., S.W. Drew, "Bioprocess technology: fermentation, biocatalysis, and bioseparation", Wiley-Blackwell, John Wiley & Sons Inc., 1999, vol 1 – 5.

Jones, D.T. and Woods D.R., 1986, Acetone-Butanol Fermentation Revisited. Microb. Rev. Dec, 484-524

Linden, J.C., Moreira A.R. and Lenz T.G., 1986, Acetone and butanol, p. 915-931. In "Comprehensive biotechnology. The principles of biotechnology: engineering consideration", Eds. Cooney C.L. and Humphrey A.E., Pergamon Press, Oxford

Napoli, F., Olivieri G., Marzocchella A. and Salatino P., 2009, An Assessment of the Kinetics of Butanol Production by *Clostridium acetobutylicum*, Int. J. Chemical Reactor Engineering 7, A45

Welsh, F.W. and Veliky I.A., 1984, Production of acetone butanol from acid whey, Biotechnol. Lett. 6, 61-64

Qureshi N, B A Annous, T C Ezeji, P Karcher and I S Maddox, "Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates", Microbial Cell Factories, 2005, vol. 4**,** 24