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Pervaporative recovery of ABE during continuous cultivation: Enhancement of performance

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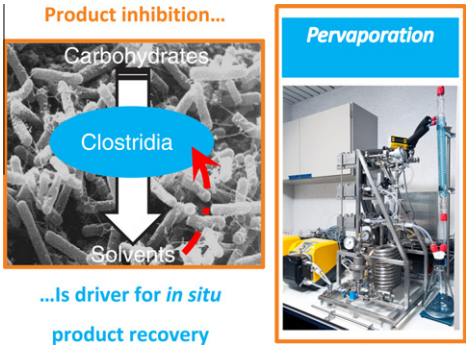
HIGHLIGHTS

- Elevated carbohydrate concentrations lead to substrate inhibition in the first bioreactor.
- High solvent titers in the second bioreactor were beneficial for pervaporation performance.
- Hybrid design combining pervaporation and conventional downstream processing was proposed.
- Trade-off between solvent productivity and energy consumption of pervaporation.

GRAPHICAL ABSTRACT



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ABSTRACT

Acetone, butanol and ethanol were produced in a continuous two-stage fermentation integrated with pervaporation using freely suspended cells of *C. acetobutylicum* ATCC 824. PDMS composite pervaporation membranes were directly coupled to the second fermentor which lead to decreased solvent titers. Overall productivity was increased from 0.45 g L^{-1} h^{-1} to 0.88 g L^{-1} h^{-1} when increasing the carbohydrate concentration in the feed from 60 to 120 g L^{-1}. The highest overall productivity of 1.13 g L^{-1} h^{-1} was achieved when increasing the carbohydrate concentration further to 150 g L^{-1} even though productivity decreased significantly in the first fermentor due to substrate inhibition. In this phase that lasted 200 h, the average flux reached 0.621 kg m^{-2} h^{-1} and the total solvent concentration in the permeate was 202 g L^{-1}. High solvent titers in the second fermentor were beneficial for the performance of the pervaporation unit leading to higher fluxes and total solvent concentrations in the permeate.

1. Introduction

The ABE fermentation using clostridial strains has been widely studied before. At the first half of the twentieth century, two thirds of the n-butanol was produced biotechnologically before it was gradually replaced in the 1950s and 1960s with the more competitive petroleum derived n-butanol. A first revival in biobutanol research occurred after the oil crisis in 1973. Today, large scale butanol fermentation is poised for re-introduction. The depletion of today's fossil fuel stocks, the fluctuations in fossil fuel price and security of energy sources are the impetus of the current revival in biobutanol production as substitute for petroleum-derived commodity products and fuels. N-butanol is an important commodity chemical used as a solvent while it is also converted to acrylates, ethers and butyl acetate. It provides a viable commercial market outlet that allows for biobutanol production to gain a foothold (Harvey and Meylemans, 2011). In addition, biobutanol holds great promise as a second-generation biofuel. It has a considerably higher combustion value than ethanol and its chemical properties allow blending in fuels more readily than ethanol, therefore it can

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Nomenclature

ABE	acetone–butanol–ethanol
A	acetone
B	butanol
D	dilution rate [h^{-1}]
E	ethanol
J	flux [$g\ m^{-2}\ h^{-1}$]
P	solvent productivity [$g\ L^{-1}\ h^{-1}$]
PDMS	polydimethylsiloxane
PTMSP	poly[1-(trimethylsilyl)-1-propyne]
S	glucose consumption [$g\ L^{-1}\ h^{-1}$]
VFA	volatile fatty acids [$g\ L^{-1}$]
x	feed weight fraction [–]
Q_E	effluent flow rate [$kg\ h^{-1}$]
Q_P	pervaporation flow rate [$kg\ h^{-1}$]
y	vapor weight fraction [–]
$Y_{P/S}$	solvent yield [$g_{solvents}\ g_{glucose}^{-1}$]

Greek symbols:

α	separation factor (–)

be called a genuine "drop-in" biofuel. The economics are hampered for this competitive market by two important bottlenecks: the high cost of the substrates and butanol toxicity leading to cell inhibition, low product accumulation, low space-time-yields and high purification costs (García et al., 2011).

An interesting feature of the ABE fermentation is that most clostridia are able of utilizing many different carbohydrates (including pentoses such as xylose). Therefore, hydrolysates from, amongst others: woody biomass (derived from short rotation coppice e.g. poplar and willow), bamboo, switchgrass, Miscanthus, paper and pulp industry can be used to decrease substrate costs.

For low-value, high volume products continuous production processes are often the only feasible option to decrease the production costs due to decreases in down-time and reduced lag phase periods in the fermentors. However, the increased risk of microbial contamination during prolonged fermentation periods together with *susceptibility* of the process to feedstock variations is a disadvantage. Contamination with lactic acid bacteria has been reported frequently in Russian ABE plants (Zverlov et al., 2006) and lead to lower than maximal yields of the fermentation. Bacteriophage infections also occurred in ABE fermentation plants. Rigorous sterilization schemes can prevent these infections, but complete sterilization is only possible for bioreactors up to 3000 m^3 (Heinzle et al., 2006).

The ABE fermentation is biphasic. A high growth rate accompanied by acetic and butyric acid formation in the first acidogenic phase is followed by decreased growth rate and solvent formation in the second solventogenic phase with a concomitant decrease in organic acid concentration. Continuous two-stage fermentations using *Clostridium acetobutylicum* have been amply described in literature on laboratory scale and lead to high solvent titers in the second (solventogenic) fermentor and higher overall productivities compared to batch fermentations. Solvent titers in the first (acidogenic) fermentor are kept at modest or subinhibitory concentrations preventing metabolic oscillations that are known to occur in one stage continuous conversions (Bahl et al., 1982; Godin and Engasser, 1990; Lai and Traxler, 1994; Mutschlechner et al., 2000).

Productivity and solvent titers dictate bioreactor and downstream processing cost respectively. For fermentations using freely suspended cells, the maximal growth rate is determined amongst others by temperature, product inhibition and the medium composition. Studies on the effects of vitamins (using synthetic medium) have indicated that only biotin and 4-amino benzoic acid (PABA) are essential vitamins for growth and solvent production (Soni et al., 1987) and that PABA was the only limiting component in the mineral medium developed by Monot et al. (1982).

The concentration of carbohydrates in the feed for clostridial ABE fermentations is limited to ca. 60 $g\ L^{-1}$ by butanol inhibition of the microorganisms. In any process, high feed concentrations are desirable to minimize the process flows and improve the water balance. This can be achieved by integrating the fermentation with *in situ* product recovery technologies where the generated solvents are removed from the fermentation broth when being formed. *In situ* gas stripping (Xue et al., 2012), extraction (Bankar et al., 2012), adsorption (Saravanan et al., 2010), perstraction (Qureshi and Maddox, 2005) and pervaporation (Liu et al., 2011; Van Hecke et al., 2012; Wu et al., 2012) have been investigated to attempt to alleviate product inhibition. Based on energy requirements of the recovery step, adsorption and hydrophobic pervaporation were identified as most promising unit operations for *in situ* product recovery of n-butanol (Oudshoorn et al., 2009).

The driving force for selective mass transport during pervaporation is achieved by maintaining a vacuum or applying a sweep gas at the permeate side of the membrane.

Two objectives were set in the current manuscript. A first design objective was to obtain a permeate from the pervaporation unit containing high total solvent concentrations (>20 wt.%) using membranes exhibiting stable long-term performances. Therefore, the kinetics of a two-stage system was studied where the second fermentor was coupled to a pervaporation unit using polydimethylsiloxane composite membranes. To reach the desired total solvent concentrations in the permeate the second bioreactor was operated at rather high (inhibitory) solvent concentrations during the steady state to increase the driving force for pervaporation. A second objective was to enhance overall solvent productivities for the integrated experiment outperforming previously published values using freely suspended cells directly coupled to pervaporation as ISPR (Qureshi and Blaschek, 1999; Van Hecke et al., 2012; Wu et al., 2012). A hybrid process for down stream processing combining steam stripping and pervaporation is conceptually presented based on the experimental results.

2. Methods

2.1. Culture and inoculum preparation

C. acetobutylicum strain ATCC 824 (Belgian co-ordinated collections of micro-organisms, BCCM) was used in all experiments. The protocols for preparation of stock culture, seed culture and inoculation of fermentors are described in Van Hecke et al. (2012).

The medium used for the fermentations is slightly different from the previous study and contained (for 1 L) 0.01 g NaCl, 2.2 g ammonium acetate, 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.01 mg biotin, 3 mg *p*-aminobenzoic acid (PABA), 0.2 g $MgSO_4{\cdot}7H_2O$, 0.01 g $MnSO_4\ H_2O$, 11.1 mg NH_4Fe citrate, 60 g glucose and 3 g yeast extract. The medium containing all components was prepared and filter-sterilized using a 0.2 μm Supor Membrane (VacuCap Filter, Pall Corporation, Port Washington, NY, USA).