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CONTINUOUS PRODUCT RECOVERY BY IN-SITU GAS STRIPPING/ CONDENSATION DURING SOLVENT PRODUCTION FROM WHEY PERMEATE USING CLOSTRIDIUM ACETOBUTYLICUM

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SUMMARY

The use of in-situ gas stripping for the removal of toxic butanol from a batch fermentation using Clostridium acetobutylicum P262 has been examined. A cold trap was used to recover the butanol. Significant increases in the lactose utilization rate and solvents productivity were obtained.

INTRODUCTION

Product inhibition limits the attainable products concentration in the acetone:butanol:ethanol fermentation. Generally, solvents concentrations of 15-20g/l are obtained, butanol being the most inhibitory product (Van der Westhuizen et al., 1982; Maddox, 1982). Continuous fermentation technologies involving immobilized cells (e.g. Schoutens et al., 1985; Ennis et al., 1986) or cell recycle (Afschar et al., 1985) are being developed to intensify the process, and these are sometimes integrated with techniques for in-line continuous solvents (butanol) removal by pervaporation (Groot et al., 1984) or the use of selective adsorbents (Larsson et al., 1984).

An alternative might be to use gas-stripping to remove solvents from the fermentation broth continuously, with solvent recovery from the vapour phase being achieved by condensation in a cold trap or by the use of a molecular sieve.

Raoult's law, modified by using activity coefficients to account for non-ideality, was used to calculate the minimum theoretical stripping-gas usage rate for such an operation. A butanol concentration of 5g/l and a butanol removal rate of 0.30g/l.h were assumed, since these values are typical of those obtained during a batch fermentation of sulphuric acid casein whey permeate medium using C. acetobutylicum P262 (Ennis & Maddox, 1986). The following assumptions were also made:

1. Equilibrium is attained between the stripping gas bubbles and the butanol in solution.

2. A butanol vapour pressure activity coefficient of 59.5 was used, calculated by the method given by Fieldes (1976) for a 5g/l butanol solution at 34°C. This activity coefficient agrees very closely with reported values (Butler et al. 1933).
3. Ideal gas law behaviour.
4. No effect of medium salts or other solvents on the butanol vapour pressure.
5. Operation at atmospheric pressure.
6. Constant butanol concentration.
7. The stripping-gas is butanol-free.

Such a calculation predicts a stripping-gas usage rate of 1.34 l/l.min.

The purpose of the present work was to demonstrate the efficacy of using gas-stripping, with solvents recovery by condensation, for continuous recovery during a batch fermentation. An initial experiment was performed with a model solvent-water solution to test the theory and assumptions.

MATERIALS AND METHODS

Microorganism

C. acetobutylicum P262, from Professor D R Woods (University of Cape Town, South Africa), was kept as a spore suspension in sterile distilled water at 4°C.

Model Solutions & Medium

A model solution containing 5g/l, 2g/l and 1g/l of n-butanol, acetone and ethanol respectively was prepared using distilled water. All solvents were reagent grade.

Spray-dried sulphuric acid casein whey permeate, from the New Zealand Dairy Research Institute (Palmerston North, N.Z.) prepared as described by Matthews et al. (1978), was reconstituted to 55g/l and yeast extract (Difco Laboratories, Detroit, Michigan, U.S.A.) and lactose (BDH Ltd, Palmerston North, N.Z.) were added to final concentrations of 5g/l and 25g/l respectively. The medium was adjusted to pH 6.5 prior to autoclaving, and from pH 5.4 to pH 6.0 after autoclaving, using aqueous ammonia.

Experimental Apparatus

A schematic diagram of the integrated batch fermenter - gas stripping apparatus is depicted in Figure 1. A Multigen F2000 fermenter (New Brunswick Scientific Co. Inc., New Brunswick, New Jersey, U.S.A.) equipped with a 2 litre glass vessel of 1.2 litre working volume is used. A 5M ammonia solution is used for pH control, and antifoam addition is controlled via a conductivity probe. When stripping commences, oxygen-free nitrogen gas is introduced into the fermenter via the agitation shaft. Exit gas is circulated to the condenser apparatus (10 litre), containing a cold finger (approx. -60°C), prior to being recirculated to the fermenter using a twin-head Masterflex peristaltic pump (Cole Palmer Instrument Co., Chicago, Illinois, U.S.A.). A gas bleed valve is located in this recycle line (ca 5-10ml/min). Solid CO_2 is used in the cold finger to recover the solvents from the vapour.

Fermentation

After autoclaving (121°C/15min), the fermenter was cooled to 34°C in a waterbath, with sterile oxygen-free nitrogen gas

being passed across the medium surface. The fermenter was inoculated (2%v/v) with a culture of highly motile cells prepared as described by Ennis & Maddox (1986). The gas stream flow was maintained after inoculation until visible gassing due to bacterial growth was observed. The pH was maintained manually at pH 6.0 ± 0.1 for 4 hours prior to assembling the fermenter vessel onto the fermenter unit. The pH was then automatically maintained at pH 6.0 ± 0.1 for a further 4.5 hours, after which time it was not controlled. The culture was agitated at 50 rpm and maintained at 34°C. After 24 hours, nitrogen gas was used to flush the gas stripping - condenser circuit prior to it being introduced into the fermenter culture. Agitation was stopped at this time.

Analyses

Solvents and acids were determined by gas chromatography (Shimadzu, Model GC-8A) using a flame ionization detector (220°C) and a column of Porapak Q. The carrier gas (N_2) flow rate was 30ml/min and the injector and column temperatures were 220°C and 200°C respectively. Samples were acidified using orthophosphoric acid. An internal standard of sec-butanol was used. Lactose was analyzed by HPLC (Ennis & Maddox, 1985).

RESULTS AND DISCUSSION

Experiments with the model fermentation solution were carried out in the experimental apparatus (Fig 1) at 34°C and an agitation speed of 50 rpm. A stripping-gas flowrate of 2.7 l/l.min was required to achieve an average butanol removal rate of 0.30g/l.h over a 12h period. Mass balance data for this run are given in Table 1, and generally agree within experimental error. The selectivity of the butanol removal by gas-stripping/condensation was found to be 19.3 using the equation proposed by Groot et al. (1984). The slightly higher stripping-gas flowrate required than theoretically predicted was expected as assumptions 6. and 7. were approximations only. In particular the butanol concentration was not constant, and the instantaneous removal rate decreased as the butanol was removed. In this instance, however, an assessment of the gas integrity of the experimental apparatus was necessary, so that the overall solvent productivity could be determined for the fermentation experiment.

Table 1: Mass balance data from gas-stripping/condensation recovery of a model fermentation solution

	Butanol	Acetone	Ethanol	Water
	(grams)			
Initial	6.14	2.27	0.80	1200
Final	2.80	1.24	0.53	1125
Condenser	3.19	0.80	0.29	70
% Recovery	97.6	89.8	105.0	99.5

The fermentation profile for the gas-stripping/condensation solvents recovery experiment at a stripping gas flowrate of 2.7 l/l.min is given in Figure 2. The concentration data are corrected for water removal during gas-stripping. Parameters derived from this experiment are given in Table 2 along with corresponding data from a previously reported fermentation without gas-stripping. Significantly, a higher lactose utilization rate and a greater extent of lactose utilization were found in the gas-stripping experiment. A higher solvents productivity was obtained by this integrated process due to in-situ toxic butanol removal.

Table 2: Summary of fermentation parameters for a control (no gas-stripping) and gas-stripping/condensation solvents recovery from a batch fermentation using sulphuric whey permeate medium and C. acetobutylicum P262.

	Control[a]	Gas-stripping/ Condensation recovery[b]
Fermentation time, h	50	52
butanol, g/l	8.1	11.0
acetone, g/l	2.9	3.9
ethanol, g/l	0.3	0.8
lactose utilized, g/l	29	58.3
yield, g solvents/g lactose utilized	0.39	0.27
overall fermenter productivity, g solvent/l.h	0.22	0.31
maximum observed lactose utilization rate, g/l.h	1.1	2.3

[a] Ennis & Maddox (1986)
[b] assumes 100% recovery

A shift to further acid production occurred after 32h, resulting from a combination of high pH (5.8) and low residual lactose (23 g/l) which is known to favour acid production (Ennis & Maddox, 1986). This result also explains the lower final yield compared with the control fermentation, and suggests that continuous lactose feeding during gas-stripping could possibly be used for extended-batch operation. A butanol removal rate of 0.45g/l.h and a butanol removal selectivity of 23.4 were obtained in this experiment, selectivity being comparable to that obtained in an integrated fermentation-pervaporation process (Groot et al., 1984). The rise in pH at the commencement of gas-stripping (Fig 2) can be attributed to the lowering of dissolved fermentation gases concentration (CO_2+H_2) in the broth. This may be important since an inverse relationship between the dissolved hydrogen gas concentration and the final solvent (butanol) concentration has been demonstrated (Doremus et al., 1985; Yerushalmi et al., 1985). When the dissolved hydrogen concentration is maximised (no agitation, hydrogen head-space

pressurization of the fermenter) solvent production is generally enhanced due to a redirection in the electron flow favouring butanol production (Maddox et al., 1981). Conversely, during batch fermentations with increased carbon dioxide head-space pressure (0-690 kPag), lower solvent concentrations and substrate utilization rates were observed (Klei et al., 1984).
Integration of this in-situ recovery process with immobilized cells and cell recycle may give rise to higher fermentation productivities. Such processes would require in-line stripping since large stripping-gas volume usage rates would be impractical. Such an integrated process for ethanol removal involving immobilized cells of K. fragilis NRRL 2415 has been reported (Dale et al., 1985).

In conclusion, this study has demonstrated that in-situ gas-stripping with solvent recovery by condensation can be used to selectively remove toxic butanol from model fermentation solutions and fermentation broths. In the latter, a significant increase in fermentation performance is achieved.

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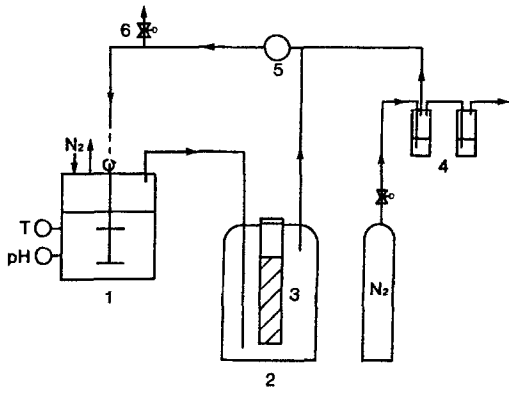

Figure 1

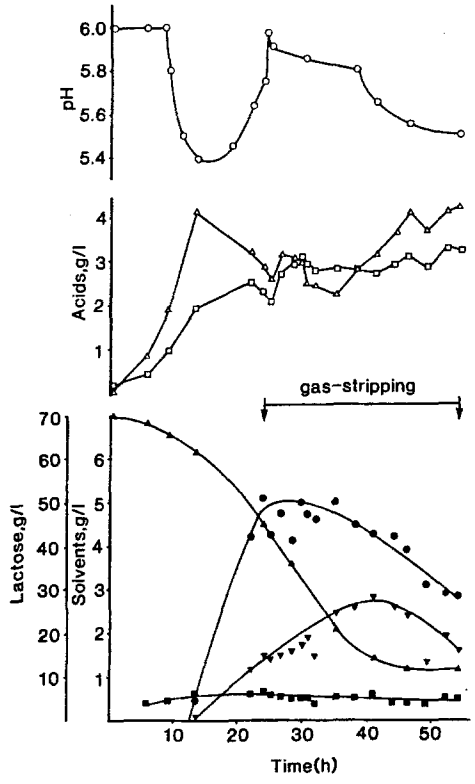

Figure 2

Figure 1: Schematic diagram of the integrated batch fermenter-gas stripping apparatus. Numbered equipment: 1 fermenter; 2 condenser; 3 cold finger; 4 gas inlet/outlet; 5 gas recycle pump; 6 gas exit.

Figure 2: Fermentation profile for the gas-stripping/condensation solvents recovery from a batch fermentation using sulphuric whey permeate medium and C. acetobutylicum P262.

pH, **O** ; butyric acid, **Δ** ; acetic acid, **□** ; lactose, **▲** ; butanol, **●** ; acetone, **▼** ; ethanol, **■** .