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Production of solvents (ABE fermentation) from whey permeate by continuous fermentation in a membrane bioreactor

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Abstract. A continuous bioreactor where cells were recycled using a cross-flow microfiltration (CFM) membrane plant was investigated for the production of solvents (ABE fermentation) from whey permeate using Clostridium acetobutylicum P262. A tubular CFM membrane plant capable of being backflushed was used.

The continuous fermentations were characterized by cyclic solventogenic and acidogenic behaviour, and ultimately degenerated to an acidogenic state. Steady-state solvent production was obtained for only short periods. This degeneration is attributed to the complex morphological behaviour of this strain of organism on this substrate.

It is postulated that to achieve steady-state solvent production over extended periods of time, it is necessary to maintain a balance among the various morphological cell forms, i.e. acid-producing vegetative cells, solvent-producing clostridial cells, and inert forms, e.g. spores.

1 Introduction

The use of membrane processes in intensified continuous fermentation processes is receiving increased attention. One such membrane process is tangential-flow filtration, more commonly called cross-flow microfiltration (CFM). In this instance, cells can be recovered from a fermentation broth using microporous membranes and recycled back to the fermenter in order to increase the biomass concentration and the fermenter productivity. Cells are retained within a closed loop, permitting higher dilution rates to be used than in conventional continuous free-cell fermentations where the dilution rate must be less than the maximum growth rate of the organism. The use of these so-called membrane bioreactors has recently been reviewed [1, 2, 3].

Potential advantages associated with the use of membrane (CFM) bioreactors are: productive cells are returned to the fermenter for re-use; greater biomass concentrations are achievable due to the availability of fresh nutrients in the feed medium, facilitating improved volumetric productivities; and inhibitory products can be removed from the cell-free filtrate stream, possibly more easily than when cells are present, prior to its return (partial) to the fermenter, giving rise to even greater productivities. Potential disadvantages may include: the need for expensive membrane plant; membrane fouling, reducing the operational period of continuous fermentation; the increase in biomass concentration may not give proportional increases in productivity due to diffusional limitations or retarded growth or metabolic activity; and the process may be complex and difficult to operate for extended periods. Additionally, comparatively little is known about the fermentative behaviour and kinetic parameters of concentrated cell suspensions in continuous culture for extended periods.

Continuous acetone : butanol : ethanol (ABE) solvents production using CFM membrane cell-recycle continuous reactors from semi-synthetic medium containing glucose using C. acetobutylicum, ATCC 824, has been described [4, 5, 6]. Significantly higher productivities and comparable solvent concentrations were reported by these workers compared to batch fermentations using freely suspended cells.

Whey and deproteinated whey (permeate) have been reported as substrates for the ABE fermentation, in both batch and continuous immobilized cell processes [7–13]. However, the use of this technical substrate in a continuous process using CFM mediated cell recycle has not so far been reported.

The purpose of the work described here was to investigate the continuous production of solvents from whey permeate using cell-recycle by CFM and to compare this process with batch and continuous immobilized cell processes using the same substrate. The organism used was C. acetobutylicum, P262, a highly differentiated strain of industrial origin. In preliminary work, an evaluation of a Plate and Frame, Hollow Fibre and Tubular CFM apparatus was conducted [14]. The Tubular CFM apparatus was the most suitable of those tested and was chosen for the present work.

2 Materials and methods

2.1 Organism

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

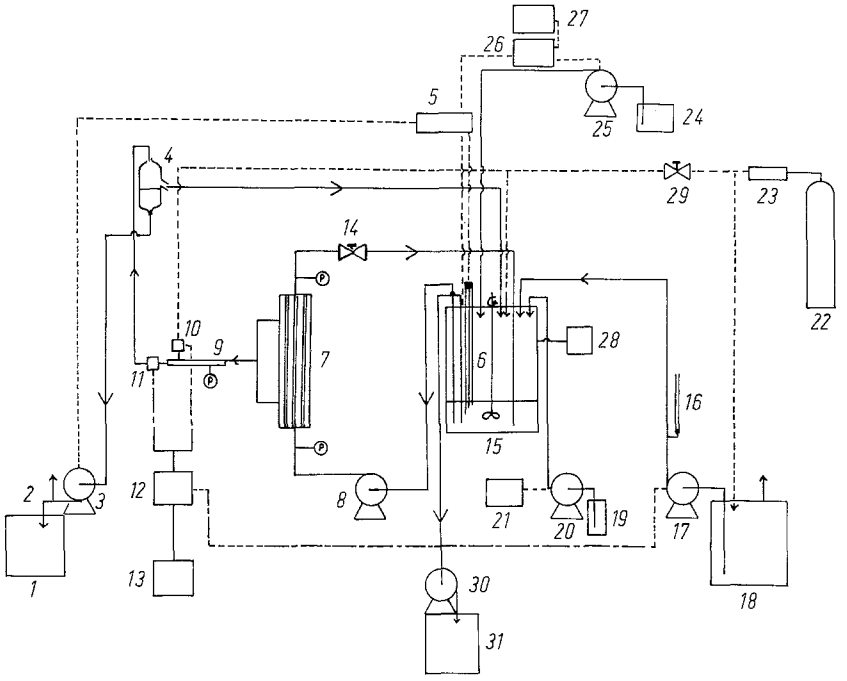

Fig. 1. A schematic diagram of the fermenter and ancillary equipment used for continuous fermentation using external cell recycle with a Tubular cross-flow microfiltration unit. *1* effluent container, *2* sampling, *3* effluent pump (filtrate), *4* filtrate holdup/overflow, *5* conductivity level control, *6* conductivity probe, 7 Ceraflo Tubular CFM Unit, *8* CFM recirculation pump, *9* pressure tube for backflush, *10* solenoid valve: nitrogen gas pulse, *11* solenoid valve: filtrate pressure buildup, *12* electronic sequence control, *13* electronic timer, *14* CFM backpressure valve, *15* fermenter, *16* medium feed flow measurement, *17* medium feed pump, *18* medium reservoir, *19* antifoam reservoir, *20* antifoam pump, *21* antifoam electronic timer, *22* nitrogen gas supply, *23* gas filter, *24* ammonia solution reservoir, *25* ammonia solution pump, *26* pH controller, *27* pH recorder, *28* temperature recorder, *29* nitrogen gas regulator, *30* biomass removal pump, *31* biomass effluent container

2.2 Materials

Spray-dried cheese whey permeate was obtained from the New Zealand Dairy Research Institute. The fermentation medium contained whey permeate (60 kg/m^3) and yeast extract (5 kg/m^3, Difco Laboratories, Detroit, Michigan, USA) dissolved in distilled water. The medium was sterilized by autoclaving at 121 °C for 20 min.

The semi-synthetic medium containing lactose, which was used for the commencement of continuous experiments using whey permeate medium, contained the following (in kg/m^3): lactose, 65; yeast extract, 6; ammonium acetate, 2; NaCl, 1; KH_2PO_4, 0.75; K_2HPO_4, 0.75; cysteine HCl $\cdot H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot 7H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.01. The semi-synthetic medium containing glucose contained (in kg/m^3): glucose, 70; yeast extract, 5; plus other components at the concentrations used in the semi-synthetic medium containing lactose.

2.3 Inoculum preparation

Spore stock of C. acetobutylicum, P262, (0.1 cm^3) was transferred to 20 cm^3 of Cooked Meat Medium (Difco Laboratories) supplemented with lactose (10 kg/m^3) and heat-shocked at 75 °C for 2 min, then cooled in an ice-water bath for 1.5 min prior to incubation at 34 °C in an anaerobic jar. After 18–24 h incubation, vigorous gassing and a highly motile culture were observed. This culture was used to inoculate 100 cm^3 of semi-synthetic medium containing lactose which was incubated at 34 °C in an anaerobic jar for 24 h. The inoculum ratio was varied between 0.1 to 1.0% (v/v), so that the most highly motile culture could be selected by microscopic examination and used as the inoculum for the final fermentation (1.0% v/v inoculum).

2.4 Experimental apparatus

The fermentation apparatus used is schematically depicted in Fig. 1. The fermenter vessel used was a $7 \cdot 10^{-3}$ m^3 pyrex glass vessel (New Brunswick Scientific Co., New Brunswick, New Jersey, USA) of $1.1–1.2 \cdot 10^{-3}$ m^3 working volume. Agitation during the initial batch fermentation was maintained at 50 min^{-1} and was provided by a single flat blade [4] impeller situated 6 cm above the base of the fermenter. Agitation was stopped when continuous fermentation with cell recycle was commenced.

Feed medium supply, fermenter culture removal from the fermenter, and filtrate removal from the system, were accomplished using variable speed Masterflex peristaltic pumps (Cole Palmer Instrument Co., Chicago, Illinois, USA).

Antifoam solution (10% (v/v) Bevaloid 5901, Bevaloid Ltd, Levin, New Zealand) was manually or automatically added via a peristaltic pump using an electronic timer (Biotechnology Department, Massey University) to control the duration of, and interval between, additions.

A Ceraflo assymetric ceramic microfilter cartridge (0.1 m^2 membrane area) was obtained from the Norton Company (Worcester, Massachusetts, USA). The CFM cartridge contained 28 tubes of 0.28 cm diameter constructed of sintered alumina with a surface pore size of 0.45 μm. The maximum flowrate was 0.05 m^3/min.

The membrane cartridge was constructed of 316 L stainless steel and equipped with standard sanitary clamp fittings to connect the cartridge to the feed pump. Teflon fittings were used on the filtrate outlets.

An Amicon lab-scale variable speed motor (Amicon Corporation, Model DC 10LA) fitted with Albin gear pump unit (Albin Ltd, Georgia, USA, Type RBS-4-83150) was used to feed the CFM cartridge. This pump had a maximum flowrate capacity of $30 \cdot 10^{-3}$ m^3/min at 170 kPa and a maximum discharge pressure of 310 kPa. The CFM feed and return lines were placed below the fermenter culture level.

The pressure differential across the membrane was maintained at 14–17 kPa. A ball valve on the concentrate outlet was used to provide backpressure for filtrate flow. Flow through the CFM cartridge was maintained as high as practically possible for continuous operation of the apparatus. The initial average transmembrane pressure drop (inlet and outlet pressure/2) was maintained at 40 kPa to minimize the initial fouling of the membrane and to provide sufficient filtrate flux, so that the overall dilution rate could be maintained. A tangential velocity of 0.75 m/s was the maximum flow that could be practically utilized with this particular experimental apparatus.

A three-contact conductivity probe plus conductivity level controller apparatus was used to control the culture level in the fermenter [14]. The culture volume was maintained constant in the apparatus by maintaining the sum of the culture (biomass) removal rate and filtrate removal rate equal to the medium feed rate (overall volumetric dilution rate). Filtrate was removed from the fermentation system using a peristaltic pump connected to the conductivity level controller apparatus using a 50 cm^3 reservoir with overflow arm to return filtrate back to the fermenter. Hence, the overall dilution rate could be varied up to the maximum capacity of the filter device. The total system volume (fermenter plus CFM apparatus) was $1.8 \cdot 10^{-3}$ m^3.

During continuous operation the membranes were cleaned by a technique known as backflushing. This membrane self-cleaning mode of operation is designed to reduce membrane fouling and facilitate long term operation. This was controlled using an electronic sequence timer (Biotechnology Department, Massey University). A separate electronic timer controlled the interval between backflush operations. Once activated, an energized open solenoid valve (Honeywell Inc., Skinner Valve Division, Connecticut, USA) was closed, blocking the filtrate flow and allowing the pressure to rise in the filtrate side of the membrane cartridge and equilibrate at the transmembrane pressure (average of the inlet and outlet pressure).

Concurrently the feed medium pump was stopped. After allowing the pressure to increase on the filtrate side a second solenoid valve was opened (0.5–2.0 s) and a nitrogen gas purge (140–205 kPa) was used to force filtrate in the filtrate line back into the membrane cartridge. This valve was then shut and after a 10 s delay the other solenoid valve reopened to allow filtrate to flow back to the fermenter. After a further delay sequence (1–5 min) the feed medium pump was restarted. This delay enabled the membrane cartridge to remove by filtration that volume of filtrate forced by backflushing back into the concentrate side of the membrane and hence into the fermenter.

Whilst this backflushing operation was not optimized, it was found to contribute, in conjunction with a low initial transmembrane pressure and as high a tangential velocity as practically possible, to the maintenance of long term flux.

2.5 Fermentation

After sterilization (121 °C, 20 min), the fermenter was cooled under a nitrogen gas atmosphere. The fermenter ($4 \cdot 10^{-3}$ m^3 of semi-synthetic medium containing lactose) was inoculated and maintained at 34 °C in a thermostated water bath for 5 h prior to connecting it to the fermenter apparatus.

A batch fermentation was carried out for 24–28 h, conducted under pH control using aqueous ammonia, after which time the fermenter was aseptically connected to the CFM unit. The fermenter volume was reduced by flushing out the CFM unit to displace the hold-up volume to waste, prior to connecting it to the fermenter. Cheese whey permeate feed medium was then commenced. Culture removal was commenced at a later time. Oxygen-free nitrogen gas was swept across the culture surface during continuous operation. When it was required to maintain the culture pH above a certain value during continuous operation, this was achieved by automatic addition of 8 M NH_4OH, and the pH value was continuously recorded.

Samples of the fermenter culture for biomass determination or microscopic examination were taken from the concentrate return line to the fermenter via a bleed valve.

A simple mathematical analysis of a continuous fermentation – cell recycle system demonstrated that for steady-state production, a constant biomass concentration must be maintained. In order to achieve this, cells were bled from the reactor at a rate equal to the growth rate ($\mu = \alpha D$, where αD is a fraction of the overall dilution rate).

2.6 Analyses

Solvents and acids were determined by gas chromatography as previously described [15] and sugars were determined using high performance liquid chromatography as previously described [10].

3 Results

Preliminary experiments revealed that there was a rapid decline in CFM membrane filtrate flux when cheese whey permeate medium, which precipitated heavily during autoclaving, was used in the initial batch fermentation phase [14]. Sterile filtration of the hot medium after autoclaving, to remove the mineral precipitate, was unsatisfactory, since the subsequent bacterial growth was poor. Further, sterilization of the medium by membrane filtration was not a practical option for this work. Consequently, in order to minimize the

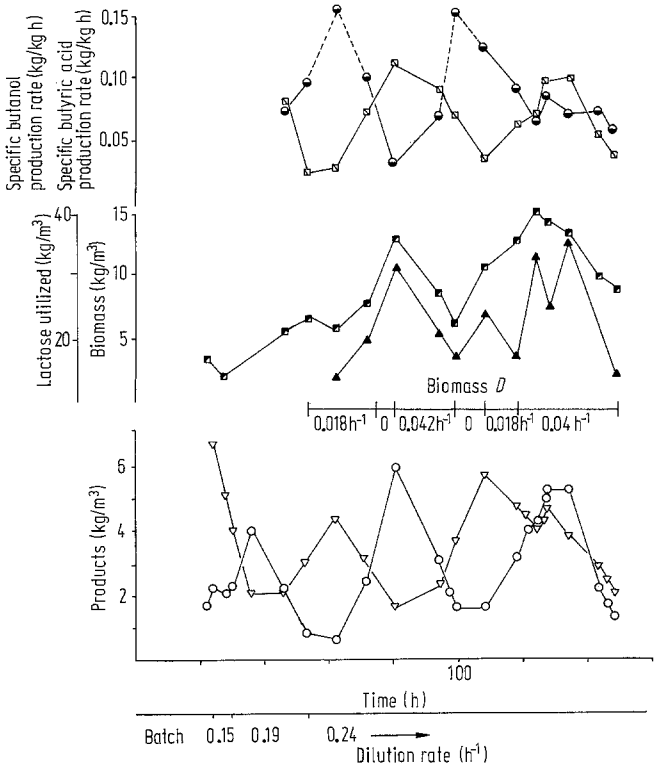

Fig. 2. Continuous fermentation profile of Run I (for experimental details, see text). ○, butanol; ▽, butyric acid; ◩, biomass concentration; ▲, lactose utilization; ⧅, specific butanol production rate; ◒, specific butyric acid production rate

initial decline in flux due to fouling, fermentations were routinely commenced using semi-synthetic medium containing lactose or glucose during the initial batch fermentation phase, prior to the commencement of the CFM membrane operation and feeding with cheese whey permeate medium. During continuous feeding of the cheese whey permeate medium, care was taken to draw-off medium from the feed medium reservoir above the settled mineral precipitate layer. Some suspended precipitate was, however, still introduced into the bioreactor. Additionally, further minerals precipitation occurred when conducting continuous fermentations under pH control at pH 5.4–5.6.

In preliminary experiments at 30 °C (data not given), pH control was not used during continuous operation and the culture was in the region pH 4.5–4.8. Biomass removal ($\alpha D = 0.01–0.03\ h^{-1}$) was utilized in order to control the biomass concentration in the region 10–15 kg/m^3. These experiments were characterized by fluctuations in both the solvents and acids concentrations and extent of lactose utilization. Steady-state operation was not achieved and solvent production was always accompanied by a high level of acid production. After a brief solventogenic period the fermentation broke down to a predominantly acidogenic state. A heterogeneous culture morphology (vegetative cells, clostridial-shaped cells, sporulating cells and some spores) was observed throughout the fermentation.

In subsequent experiments, the pH was controlled in the range pH 5.4–5.6. Solvent production and lactose utilization have been shown to be optimal in this pH region in batch fermentations using freely suspended cells of strain P262 [16]. Fig. 2 shows the fermentation course profile of Run I conducted at 34 °C with the pH controlled at pH 5.4–5.6. The biomass removal rate (αD) was based on values used in the preliminary experiments with no pH control, and which maintained the biomass concentration within a chosen range. For clarity, Fig. 2 does not show acetone, ethanol or acetic acid concentration data. Butyric acid was the predominant acid, present at 3 to 4 times greater concentration than acetic acid. The ethanol concentration was low and invariably less than 0.4 kg/m^3, while the acetone concentration profile followed that of butanol, but at approximately 2 to 3 times lower concentration.

The overall dilution rate was increased after a 24 h period of continuous operation to a value of $D = 0.24\ h^{-1}$, and this was maintained throughout the duration of the experiment. A maximum solvent concentration of 9.3 kg/m^3 (6 kg/m^3 butanol) was observed at 80 h, at a biomass concentration of 12.5 kg/m^3.

At this time, the observed specific butanol production rate (0.11 kg/(kg · h)) was maximal whilst the corresponding specific butyric acid production rate was low (0.03 kg/(kg · h)). A mixture of clostridial shaped cells and rod-shaped cells was present, with the former type predominant. The fluctuations in the biomass concentration were due to difficulties in controlling the biomass removal rate. At a biomass removal rate of $D = 0.018\ h^{-1}$ (between 53 h to 74 h and 108 h to 118 h) biomass accumulated suggesting that the growth rate was higher than $0.018\ h^{-1}$ at this culture pH. The increase in biomass concentration corresponded with the maximum observed solvents concentration. A cyclic behavioural pattern was observed; solvent production followed by acid production. The specific production rates of butanol and butyric acid also followed a cyclic pattern. The maximum observed solvent yield of 0.30 kg/kg was observed at the time of the maximum observed solvents concentration. Prior to this time and after this time the yield was lower and in the region 0.16–0.26 kg/kg. Generally, the lactose utilization pattern followed the biomass concentration pattern.

A further experiment was undertaken (Run II) under similar operating conditions (33 °C, pH 5.4–5.6) to those described for Run I, and a fermentation time course profile is given in Fig. 3. The objective in this Run was to allow the biomass to accumulate to a greater concentration than in the previous run, and to operate at a higher overall dilution rate in order to determine the solvent productivity capacity of this experimental system. Since in Run I, a biomass removal rate of a value $D = 0.018\ h^{-1}$ was shown to be lower than the growth rate under these culture conditions, this value was chosen for this experiment. The overall dilution rate was increased over a 30 h period to a value of $D = 0.30\ h^{-1}$ and this was maintained throughout the duration of the experi-

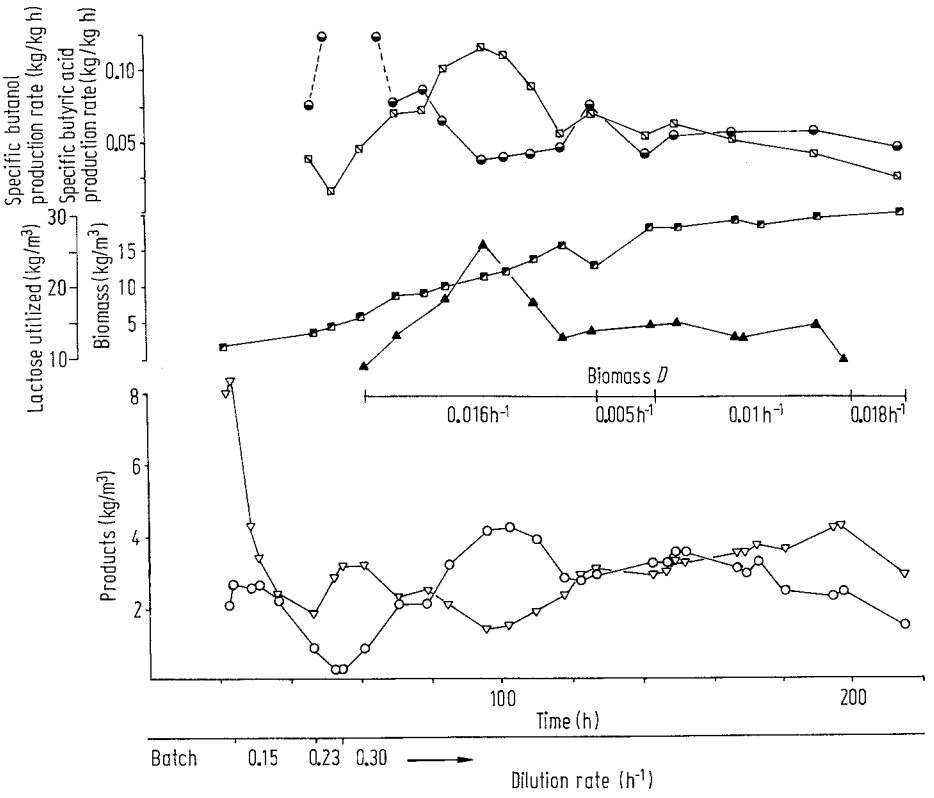

Fig. 3. Continuous fermentation profile of Run II. ○, butanol; ▽, butyric acid; ◩, biomass concentration; ▲, lactose utilization; ⧅, specific butanol production rate; ◒, specific butyric acid production rate

ment. A gradual increase in the biomass concentration was observed, reaching a maximum of 19.5 kg/m^3 after 215 h operation. Some difficulties in controlling the biomass removal rate were experienced but the fluctuations in the removal were not as pronounced as in the previous run.

A steady-state ($\pm$10%) solvent production period was obtained between 122 h and 173 h (15.3 residence times), and a total solvent concentration of 4.2–4.6 kg/m^3 was obtained representing a solvents productivity of approximately 1.32 kg/(m^3 · h). This was accompanied by a butyric acid concentration of approximately 3 kg/m^3 and an acetic acid concentration of approximately 1 kg/m^3 (data not shown). After a maximum butanol concentration and observed specific butanol production rate between 85 h and 118 h, both the solvent concentration and the specific butanol production rate decreased whilst the specific butyric acid production rate remained steady. The solvents yield during the steady-state solvent production period time was 0.31 kg/kg decreasing to 0.22 kg/kg after 191 h. Again, a heterogeneous culture morphology was observed throughout the fermentation, but no attempt was made to quantitate any particular morphological forms. The run was stopped after 215 h due to a decline in the fermentation performance.

A further experiment (Run III, fermentation time course not shown) was undertaken using cheese whey permeate medium, operating under similar conditions to Runs I and II (34 °C, pH 5.4–5.6). In this instance, however, the initial batch fermentation phase was conducted using semi-synthetic medium containing glucose. This was done in an attempt to obtain solventogenic conditions and hence a more homogeneous culture morphology than observed when commencing using semi-synthetic medium containing lactose. Continuous operation and cell recycle were commenced after 24 h and cheese whey permeate medium was used at this time at an overall dilution rate of 0.41 h^{-1}. The total solvents and acids concentrations were 9.9 kg/m^3 and 2.9 kg/m^3, respectively, and early and late-stage clostridial cells (>90%) were observed at this time. After 50 h fermentation, a mixture of clostridial-shaped cells, sporulating cells and free spores was observed.

Steady-state solvent production was achieved for approximately 4 residence times at an overall dilution rate of 0.41 h^{-1}, giving 4.90 kg/m^3, 1.94 kg/m^3, 0.29 kg/m^3, 1.50 kg/m^3, and 1.55 kg/m^3 of butanol, acetone, ethanol, butyric acid and acetic acid, respectively. This was equivalent to a solvent productivity of 2.92 kg/(m^3 · h) with a solvent yield of 0.31 kg/kg. The biomass concentration was controlled in the region 11.8–12.8 kg/m^3 using a biomass dilution rate of 0.02 h^{-1}. The specific butanol production rate and specific lactose utilization rate were approximately 0.16 and 0.80 kg/(kg · h), respectively, during this period. After this steady-state, solvent production decreased and acid production increased to 4.9 kg/m^3 butyric acid and 1.5 kg/m^3 acetic acid (butanol = 2 kg/m^3) after 162 h, and the fermentation was stopped.

4 Discussion

The objective of this work was to investigate the conditions for continuous stable solvents production from cheese whey

permeate medium, using free cells of C. acetobutylicum, P262, with external cell recycle by cross-flow microfiltration (CFM), and to compare this process with those reported using immobilized cell systems.

Initial experiments (30 °C, pH 4.5–4.8) were characterized by a brief solventogenic period after which an acidogenic state occurred. The heterogeneous culture morphology observed at these culture pH values may explain the culture degeneration, i.e. fast-growing, acid-producing vegetative rod-shaped cells were progressively selected for under these conditions [14].

In subsequent experiments (Runs I to III), operated at higher pH values (pH 5.4–5.6), the culture also broke down to an acidogenic state, generally after 120–140 h fermentation (Figs. 2 and 3). Cell growth occurred during the fermentation with a consequent increase in the biomass concentration, indicating the presence of vegetative cells. Steady-state solvent production was not achieved in Run I, and only briefly in Runs II and III.

C. acetobutylicum, P262, is a highly differentiated strain [17, 18]. In batch culture, three morphological forms were observed, and were associated with different stages of the fermentation process [17]. During acid production, actively growing vegetative cells were rod-shaped, however, during the solvent production phase, growth ceased and a morphological change occurred with vegetative cells changing to fattened cigar-shaped solvent-producing clostridial cells. The cells then sporulated and did not divide or produce acids or solvents [17]. These morphological changes have been observed in batch fermentation of sulphuric acid casein whey permeate medium using strain P262 [14]. The same morphological forms and changes as observed in batch culture, were observed during the continuous fermentations described above.

A schematic representation of this morphological cycle is given in Fig. 4. The rate of conversion, or differentiation, of one morphological form to another is designated by a reaction rate K. These non-reversible changes occur as a result of complex interactive triggering mechanisms. For example, the morphological change from vegetative cells to clostridial cells at rate K_1, involves the acid concentration (external and internal concentration?, dissociated or undissociated form?), pH, and residual sugar and nitrogen concentrations [18].

Conceptually, for the maintenance of continuous stable solvent production, the conditions must be such that the clostridial cells are maintained in a stable non-growing solvent production phase, with vegetative growth, sporulation, and cell lysis inhibited or closely controlled, i.e. a balance of culture morphology must be maintained. The degeneration of these continuous fermentations to an acidogenic state, i.e. an unbalanced culture morphology, may be due to cells not triggered initially or as the result of culture derepression where toxic products that are inhibitory to further solvent production are washed out, thus allowing further cell growth.

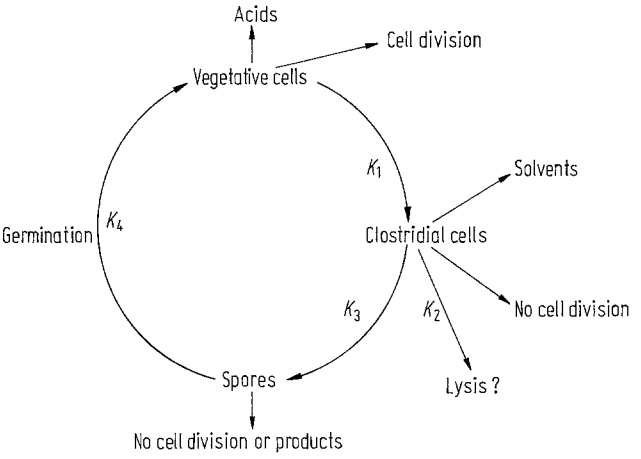

Fig. 4. A schematic diagram of the morphological changes observed during continuous fermentation by C. acetobutylicum, P262, fermenting whey permeate medium. K_1 designates the rate of conversion (or differentiation) of vegetative cells to clostridial forms. K_2 designates the rate of loss of clostridial forms due to lysis. K_3 designates the rate of sporulation of clostridial forms. K_4 designates the rate of spore germination to vegetative cells

An attempt was made in all runs to control the biomass concentration, in order to obtain steady-state solvent production conditions. This was done by removing biomass directly from the bioreactor on a continuous basis; hence solvent-producing clostridial cells were also removed. For given culture conditions, e.g. pH, medium composition, products concentration, overall dilution rate and cell concentration, acid-producing vegetative cells differentiate to solvent-producing clostridial cells at a given rate (K_1). This rate may change under different culture conditions, e.g. it may decrease as the solvent concentration increases. It is possible then, that clostridial cells are removed in the biomass bleed, at a rate greater than the differentiation rate that occurs during solvent production, i.e. $\alpha D \gg K_1$. Thus a lowering in the solvent production rate occurs, hence the solvents concentration is lowered. This in turn favours growth and an increase in acids production, with these cells in turn differentiating to clostridial forms favouring solvent production.

A cyclic solventogenic pattern can result as depicted in Figs. 2 and 3. If the biomass removal rate were maintained at a rate equal to the differentiation rate K_1, (assuming no sporulation or cell lysis) then, theoretically, control over the solvent-producing clostridial population would be possible. Whether this remained stable would depend on the balancing of environmental conditions, e.g. avoidance of toxic product concentrations. In this situation however, vegetative cells could still accumulate and the differentiation rate K_1 would be expected to change. Therefore, the maintenance of the growth rate (μ) and spore germination rate, K_4, equal to the differentiation rate of vegetative cells to clostridial cells, K_1, minus the clostridial cell lysis rate, K_2, and sporu-

lation rate, K_3, ($K_4+\mu=K_1-K_2-K_3$) is a controlling index for stable solvent production.

What affects the growth rate (μ) and the differentiation reaction rates (K) of the various morphological forms, is not fully understood. In addition to previously mentioned factors, such effects as mass transfer, e.g. diffusivity limitations in concentrated suspension, may occur under some conditions. Some cell loss (clostridial forms, and other types?) was evident in these experiments, as indicated by the turbid supernatant liquid present in culture samples centrifuged for dry weight biomass determination.

In summary, maintenance of the morphological cycle (Fig. 4), such that a balance is achieved, facilitating stable solvents production, is very complex and not fully understood. Possibly, a non-sporulating clostridial-forming mutant of strain P262, as used by Largier et al. [19] in continuous fermentation using immobilized cells, would break the cycle depicted in Fig. 4, and possibly simplify the conditions for process control. The inability in this study to obtain a true steady-state contrasts with the findings of other workers [4, 5, 6] using a different strain and substrate, suggesting that these might be important factors. Long et al. [18], for example, did not observe the different morphological forms with strain ATCC 824 as observed with strain P262.

The maximum specific butanol production rates and maximum specific lactose utilization rates obtained using cheese whey permeate medium at pH 5.4–5.6 (Runs I and II) were generally lower than those reported by other workers on media containing glucose [4, 5, 6]. One exception was the data obtained in Run III, where the steady-state specific butanol production rate and specific lactose utilization rate data are comparable with those obtained on medium containing glucose, although at a total solvent concentration of half that obtained with the latter medium. These data support the findings of Ennis and Maddox [20] that glucose is a preferred substrate to lactose. Significantly, the maximum observed specific butanol production rate in this work (0.16 kg/(kg · h) in Run III) agrees well with the value of 0.20 kg/(kg · h) obtained in batch fermentation of sulphuric acid casein whey permeate using freely suspended cells (Ennis, unpublished results).

The maximum observed solvent productivity (2.9 kg/(m^3 · h)) obtained using this bioreactor system is much lower than that routinely obtained with continuous fermentations of whey permeate using cells of strain P262 immobilized by adsorption onto bonechar [13]. In this case solvent productivities of up to 4.1 kg/(m^3 · h) have been achieved, and the system has proved to be stable over an operating period of 1400 h. Furthermore, the system is technically simpler to operate than the membrane bioreactor.

During experiments conducted with alginate-immobilized cells of strain P262 on the same substrate, productivities were comparable to that obtained in the present study, but again the system was much more stable and could be operated for extended periods [12]. As with the bonechar-immobilized cells, alginate-immobilized cells are technically simple to use. With both of the immobilized cell systems, free cells are continuously lost from the reactor, as with the cell recycle technique. However, since these systems remain stable, it may be postulated that only growing, vegetative cells are lost, while the solvent-producing clostridial forms remain immobilized. This concept is supported by microscopical examination of immobilized cells and those being lost in the reactor effluent (Ennis, unpublished results).

A quantitative assessment of the tubular CFM performance was not attempted in this study. This is undoubtedly important, since capital and operating costs of continuous CFM bioreactors are proportional to the CFM membrane area, hence average flux performance data as a function of cell concentration is a major factor determining the economic feasibility of the application of CFM to continuous free-cell fermentation processes. Such data are most likely to be case specific, relating to the rheological properties of a given cell-medium suspension and the hydrodynamic properties of the CFM plant.

This assessment would involve the examination of a large number of process variables, e.g. transmembrane pressure, tangential velocity, membrane porosity, pore distribution, biomass concentration, cleaning and sterilization procedures, and backflushing frequency, etc., for their effects on filtrate flux and membrane life with time. Also, it should be noted that the hydrodynamic properties of the small laboratory scale CFM unit are not necessarily reproduced in a larger manufacturing CFM unit. In this laboratory-scale study, some scale-down was necessary.

The tubular CFM unit was operated at a tangential velocity of approximately 10% of that achievable, hence considerable flux increases would be expected over that achieved in this work if the unit were operated at a higher tangential velocity. Such operation was not achievable in this particular instance due to the requirements for fermenter vessel level control (excessive turbulence) and the damage done to cells at higher flowrates due to the pump action.

The decline in filtrate flux with time may be attributed not only to the rheological properties and concentration polarization effects of the culture, but also to the intrinsic fouling nature of the fermentation medium per se due to membrane-solute interactions. The presence of minerals (ionic or colloidal form) in whey permeate will cause membrane fouling, the nature and extent of which is most likely to be case specific.

It is concluded that further studies are required to demonstrate the full potential of this bioreactor type for the ABE fermentation using a substrate of whey permeate. The lower solvent productivity obtained with this bioreactor compared with continuous fermentations using immobilized cells has been explained by a complex morphological behaviour that may be strain and/or medium specific. Pilot plant studies are now required to provide the necessary operational data to allow for a full technological comparison of the alternative types of bioreactors [2].

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References

1. Cheryan, M.; Mehaia, M. A.: Membrane bioreactors. Chem. Technol. 16 (1986) 676–681
2. Ripperger, S.; Schulz, G.: Microporous membranes in biotechnical application. Bioprocess Eng. 1 (1986) 43–49
3. Strathmann, H.: Membranes and membrane processes in biotechnology. Trends Biotechnol. 3 (1985) 112–118
4. Afschar, A. S.; Biebl, H.; Schaller, K.; Schügerl, K.: Production of acetone and butanol by Clostridium acetobutylicum in continuous culture with cell recycle. Appl. Microbiol. Biotechnol. 22 (1985) 394–398
5. Pierrot, P.; Fick, M.; Engasser, J. M.: Continuous acetone-butanol fermentation with high productivity by cell ultrafiltration and recycling. Biotechnol. Lett. 8 (1986) 253–256
6. Ferras, E.; Minier, M.; Goma, G.: Acetonobutylic fermentation: improvement of performances by coupling continuous fermentation and ultrafiltration. Biotechnol. Bioeng. 28 (1986) 523–533
7. Welsh, F. W.; Veliky, I. A.: Production of acetone-butanol from acid whey. Biotechnol. Lett. 6 (1984) 61–64
8. Schoutens, G. H.; Nieuwenhuizen, M. C. H.; Kossen, N. W. F.: Butanol from whey ultrafiltrate: batch experiments with Clostridium beyerinckii LMD 27. 6. Appl. Microbiol. Biotechnol. 19 (1984) 203–206
9. Maddox, I. S.: Production of n-butanol from whey filtrate using Clostridium acetobutylicum NCIB 2951. Biotechnol. Lett. 2 (1980) 493–498
10. Ennis, B. M.; Maddox, I. S.: Use of Clostridium acetobutylicum P262 for production of solvents from whey permeate. Biotechnol. Lett. 7 (1985) 601–606
11. Schoutens, G. H.; Nieuwenhuizen, M. C. H.; Kossen, N. W. F.: Continuous butanol production from whey permeate with immobilized Clostridium beyerinckii LMD 27. 6. Appl. Microbiol. Biotechnol. 21 (1985) 282–286
12. Ennis, B. M.; Maddox, I. S.; Schoutens, G. H.: Immobilized Clostridium acetobutylicum for continuous butanol production from whey permeate. New Zealand J. Dairy Sci. Technol. 21 (1986) 99–109
13. Qureshi, N.; Maddox, I. S.: Continuous solvent production from whey permeate using cells of Clostridium acetobutylicum immobilized by adsorption onto bonechar. Enzyme Microb. Technol. 9 (1987) 668–671
14. Ennis, B. M.: PhD Thesis (1987) Massey University, Palmerston North, New Zealand
15. Ennis, B. M.; Marshall, C. T.; Maddox, I. S.; Paterson, A. H. J.: Continuous product recovery by in-situ gas stripping/condensation during solvent production from whey permeate using Clostridium acetobutylicum. Biotechnol. Lett. 8 (1986) 725–730
16. Ennis, B. M.; Maddox, I. S.: The effect of pH and lactose concentration on solvent production from whey permeate using Clostridium acetobutylicum. Biotechnol. Bioeng. 29 (1987) 329–334
17. Jones, D. T.; van der Westhuizen, A.; Long, S.; Allcock, E. R.; Reid, S. J.; Woods, D. R.: Solvent production and morphological changes in Clostridium acetobutylicum. Appl. Environ. Microbiol. 43 (1982) 1434–1439
18. Long, S.; Jones, D. T.; Woods, D. R.: Initiation of solvent production, clostridial stage and endospore formation in Clostridium acetobutylicum P262. Appl. Microbiol. Biotechnol. 20 (1984) 256–261
19. Largier, S. T.; Long, S.; Santangelo, J. D.; Jones, D. T.; Woods, D. R.: Immobilized Clostridium acetobutylicum P262 mutants for solvent production. Appl. Environ. Microbiol. 50 (1985) 477–481
20. Ennis, B. M.; Maddox, I. S.: Butanol production from lactose-hydrolysed whey permeate by fermentation with Clostridium acetobutylicum: Comparison with non-hydrolysed whey permeate. New Zealand J. Dairy Sci. Technol. 22 (1987) 75–81

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