Studies on the Interaction of Fermentation and Microfiltration Operations: Erythromycin Recovery from *Saccharopolyspora erythraea* Fermentation Broths

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Abstract: Changes in fermentation media not only affect the performance of the fermentation itself (with regard to the kinetics of biomass and product formation and the yields obtained) but also the initial product-recovery operations downstream of the fermentor. In this work, microfiltration experiments to remove *Saccharopolyspora erythraea* biomass from fermentation broth and to recover erythromycin were carried out using two fundamentally different media; a soluble complex medium (SCM) and an oil-based process medium (OBM). Small-scale batch fermentations of 14-L working volume were carried out in triplicate using both media. Broth samples were taken from each fermentation at regular intervals from the end of the exponential-growth phase onwards. These were then processed using a Minitan II (acrylic), tangential crossflow-filtration module, fitted with a single 60 cm^2 Durapore hydrophilic 0.2 μm membrane, operated in concentration mode.

The OBM fermentations produced higher titers of erythromycin but required longer fermentation times due to increased lag phases and slower maximum-growth rates. The OBM also increased the loading on the membrane; at maximum product titers residual oil concentrations of 3 $g \cdot L^{-1}$, antifoam concentrations of 2 $g \cdot L^{-1}$ and flour concentrations estimated at approximately 10 g/L^{-1} were typical. It was found that both the permeate flux and erythromycin transmission were affected by the choice of medium. The OBM had significantly lower values for both parameters (12.8 Lm^{-2} h^{-1} and 89.6% respectively) than the SCM (35.9 Lm^{-2} h^{-1} and 96.7% respectively) when the fermentations were harvested at maximum erythromycin titers. Transmission of erythromycin stayed approximately constant as a function of fermentation time for both media, however, for the OBM the permeate flux decreased with time which correlated with an increase in broth viscosity. The relatively poor microfiltration performance of the OBM medium was, however, offset by the higher titers of erythromycin that were achieved during the fermentation. The filtration characteristics of the SCM broth did not show any correlation with either broth viscosity or fermentation time. Image-analysis data suggested that there was a correlation between hyphal morphology (main hyphal length) and permeate flux (no such correlation was found for the OBM broth). Moreover, it has been shown for the OBM broth that the residual flour had a profound effect on the microfiltration characteristics. The influence of the residual flour was greater than that imposed by the morphology and concentration of the biomass. The understanding of the factors governing the interaction of the fermentation and microfiltration operations obtained in this work provides a first step towards optimization of the overall process sequence. *Biotechnol Bioeng* **69**: 429–439, 2000.
Keywords: microfiltration; fermentation medium; erythromycin; *Saccharopolyspora erythraea;* image analysis

INTRODUCTION

The growing resistance of microorganisms to existing antibiotics drives the search for new antimicrobial compounds. Recent advances in our understanding of the synthesis of the polyketide antibiotics at a molecular level have enabled the production of novel polyketide molecules from the synthetic pathways of microorganisms including *S. erythraea* (Caffrey et al., 1992; Donadio et al., 1993). To decrease the time to market of these new drugs and improve the productivity of existing processes, the factors affecting their production, recovery, and purification need to be better understood.

Changes in fermentation medium formulation provide a rapid route to increased product titers and reduced media costs (Zhang and Greasham, 1999). Studies on improving fermentation media, however, rarely take account of the effects of the changes on the downstream process. Increased production can often be compromised by the loss of product during recovery operations. In the case of crossflow microfiltration, this may occur due to a reduction in permeate flux

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or reduced transmission of the target molecule through the membrane. Several studies have observed the effects of individual media components on microfiltration operations, for example antifoams (Liew et al., 1997), and oils (Conrad and Lee, 1998). However, these investigations did not take into account the effects of these components on both the fermentation and recovery processes and the interactions between them.

Broths can consist of a number of phases, for example, a continuous-aqueous phase, solid-medium constituents, such as flour particles, and insoluble organic elements, such as oils. Oils which are added at the beginning of the fermentation and which represent a more economic source of carbon and energy than glucose, may not be totally utilized by the organism at the desired time of harvest (Stowell, 1987). It is these residual oils that can potentially cause a problem for microfiltration operations. The residual-oil droplets can block the pores on the membrane leading to a decrease in permeate flux. Conrad and Lee (1998) showed that 5% (v/v) soya bean oil in water led to an order of magnitude decrease in the steady-state flux. Solid-phase constituents such as soya flour, which is used as a complex nitrogen source, can also be present in the broth at the end of the fermentation. The effects of these insoluble solids are more difficult to elucidate because they cannot easily be separated from the biomass or residual oil for analysis.

While a high-antibiotic transmission is desirable for process economics, the transmission of micron-sized solid particles through the solid–liquid separation process can have a detrimental effect on processes further downstream. Berthold and van Kemken (1994) discussed the need for further clarification of a supernatant after solids removal by centrifugation, before passing the liquor down an adsorption column, while fermentation broth clarified by microfiltration required no pretreatment. The transmission of product across the membrane is, therefore, also of great importance when considering the suitability of the unit operation to industry. A mean-transmission percentage of 97% has previously been achieved by Antoniou et al. (1990) in simulated broth experiments. By optimizing microfiltration parameters such as permeate flux and antibiotic transmission as a function of fermentor harvest time, the most effective overall process conditions will be determined.

In this study we show how a change of medium composition affects both the production of erythromycin together with its transmission and rate of flow through a membrane used to separate the biomass from whole fermentation broths. The variation of permeate flux and erythromycin transmission as a function of fermentation time has also been investigated. As a model system we have studied the growth of *S. erythraea* (a spore forming, Gram-positive, hyphae-producing bacterium) on both a soluble-complex medium (SCM) and an oil-based process medium (OBM).

MATERIALS AND METHODS

Materials and Microorganism

The strain used in these experiments was *Saccharopolyspora erythraea* CA340 which was kindly supplied by Abbott Laboratories (Chicago, IL). Spore stocks were stored in a 20% (v/v) glycerol solution at −70°C. Chemicals were purchased from Sigma-Aldrich Company unless otherwise stated and were of analytical grade. Reverse-osmosis (RO) water was used throughout for medium preparation.

Fermentation Media and Operating Conditions

The media used were a soluble complex medium (SCM) and an oil-based medium (OBM) the components of which are given in Table I. Both have previously been used in fermentation studies within our laboratory (Heydarian, 1998; Mirjalili et al., 1999; Sarra et al., 1996). Spore stocks were initially prepared from surface cultures of *S. erythraea*, the medium used contained 2 g L^{-1} glucose, 1 g L^{-1} sucrose, 5 g L^{-1} soy peptone, 2.5 g L^{-1} yeast extract, 20 g L^{-1} agar (Technical No. 1), and 0.036 g L^{-1} EDTA. The pH was adjusted to 7.0 using 0.1*M* NaOH before autoclaving. The Petri dishes were incubated for 3 weeks at 28°C, spores were removed using a sterile loop and placed in an aqueous mixture of 20% glycerol (v/v) and 0.1% Tween 80 (v/v) and stored at −20°C. For the inoculation of the fermentor 1 mL of spore stock was thawed and added to 50 mL of nutrient broth in a 500-mL baffled, conical flask and placed in an orbital shaker at 28°C for 48 h. This culture was then transferred to 450 mL of the relevant medium (SCM or OBM) in a 2-L baffled conical flask and placed in an orbital shaker at 28°C for 30 h (SCM) or 48 h (OBM). The inoculum volume was 10% of the final volume.

The fermentations were carried out in a LH 2000 series fermentor (Inceltech Ltd., Pangbourne, UK) having a total volume of 20 L and a working volume of 14 L. The vessel had a height to diameter ratio of 2.2:1, and was fitted with a three-stage 6-bladed Rushton turbine impeller (impeller diameter one third of tank diameter and spaced at 75 mm, 155 mm, and 245 mm from the bottom plate). Four equally spaced baffles of width one tenth of the diameter were pres-

Table I. Composition of the soluble complex medium (SCM) and the oil-based medium (OBM).

Soluble complex medium (SCM)	Concentration (g L^{-1})	Oil-based medium (OBM)	Concentration (g L^{-1})
Glucose	30	Soya bean flour	30
Yeast extract	6	Rape seed oil	23
Bacto-peptone	4	Dextrin	10
Glycine	2	KH_2PO_4	1.2
$MgSO_4 \cdot 7H_2O$	0.5	PPG	2.5
KH_2PO_4	0.68		
PPG	2.5		

ent. The fermentor was sparged with air at an aeration rate of 1 vvm and impeller speeds between 750 and 1250 rpm depending on the dissolved oxygen tension (DOT). The fermentor was controlled by Adaptive Biosystems Ltd. software (Luton, UK) and data logging was carried out by RT-DAS (Acquisition Systems Ltd., Sandhurst, UK). On-line exit-gas composition was measured using an MM8-80S mass spectrometer (VG Gas Analysis Ltd., Winsworth, UK). The maximum volume removed from the fermentation for the microfiltration studies (and analysis) was 3 L to keep the level of broth above that of the top impeller. Each type of fermentation was carried out in triplicate.

Microfiltration: Rig and Operation

The Minitan II (Millipore, Watford, UK) rig was operated using a one-pump configuration as shown in Figure 1, in concentration mode. This system was chosen because of the small volume of broth that could be processed, thus enabling microfiltration studies to be carried out during the course of each fermentation. The pump used was a peristaltic Watson-Marlow Du505 (Watson-Marlow, Poole, UK). Volumetric crossflow rate was kept constant close to the maximum value of 800 mL/min^{-1} which gives a crossflow velocity of 0.66 ms^{-1} at the membrane surface. The membrane used was a 0.2 μm hydrophilic Durapore membrane. A hydrophilic membrane was chosen to minimize fouling by proteins (Zhang et al., 1998) and anti-foaming agents (Liew et al., 1997). A single-membrane plate was used with a membrane area of 6×10^{-3} m^2, which enabled processing of small amounts of broth (ca. 500 mL) taken from the fermentor throughout the growth and product-formation stages. The connecting tubing used was silicon (Altec, Hampshire, UK) with internal diameter of 6.2 mm and wall thickness of 1.6 mm. The total path length was 1.5 m giving a rig hold-up volume of 45 mL.

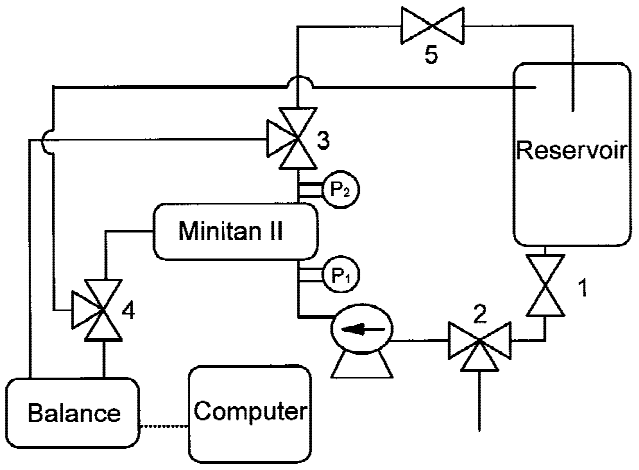

Figure 1. Schematic layout of the Minitan II acrylic rig, the inlet- and outlet-pressure gauges are denoted by P_1 and P_2, respectively. Valve 2 is to enable draining of the resorvoir; Valve 3 is to allow the volumetric crossflow to be measured; Valve 4 allows the rig to be changed from concentration to total recycle mode; and Valve 5 is the backpressure valve for controling transmembrane pressure (TMP).

The feed solution was kept homogenous in the reservoir by using an SS2 stirrer (Stuart Scientific, Newcastle, UK, TX) with a 7-cm diameter 6-bladed Rushton turbine impeller at 100 rpm. The permeate flux and the volumetric crossflow rate were measured using an electronic balance (model BB2400 Metler-Toledo Ltd., Leicester, UK). Data from the balance were recorded using Labview, the virtual instrumentation software (National Instruments, Austin, TX). For each experiment individual permeate-flux measurements were taken (over a 20-s time interval) for a period of 30 min; steady-state flux values were obtained from the final three readings and a mean and standard deviation calculated. Critical transmembrane pressure (cTMP) was taken as the point at which permeate flux reached a peak. Experiments took place at 21°C in a temperature-controlled room and no increase in the temperature of the broth due to pumping could be detected. Following each experiment, the membrane was washed in reverse osmosis (RO) water to remove solids from the membrane surface. The rig was then flushed with 5% (v/v) hypochlorite at 50°C for 1 h and 5% (v/v) Redphos Special, a phosphoric acid-based cleaner (Laporte ESD Ltd., Cheshire, UK) at 50°C for 1 h. The membrane was then rinsed with RO water and the clean-water flux was measured. If the clean-water flux was below 0.03 Lm^{-2}h^{-1}Pa^{-1} then the cleaning steps were repeated.

Analytical Techniques

Erythromycin A and Antibiotic Transmission Analysis

Erythromycin A concentration was determined by HPLC using a method described by Heydarian et al. (1998). Fermentation broth was centrifuged at 4000 rpm for 20 min (Beckman CS-6R centrifuge, Beckman, Buckshire, UK) and the supernatant was collected for analysis. For the OBM further centrifugation at 12,000 rpm and filtration through a 0.2-μm membrane were required to obtain a clear sample. Erythromycin was then concentrated by a factor of 10 using C18 bond elut cartridges (Phenomenex, Cheshire, UK). HPLC was performed on a Beckman model 126 HPLC system equipped with a Beckman model 166 UV detector at 215 nm. The column used was Polymer Laboratories PLR P-S (8 μm). Acetonitrile-10 m*M* potassium dihydrogen phosphate (pH 7) (45:55 v/v) at 1.0 mL min^{-1} was used as the mobile phase. The column temperature was controlled at 70°C with a block-column heater and the sample-injection volume was 20 μL.

Determination of the total erythromycin concentration (i.e., all forms of the antibiotic and not just erythromycin A), in broth and permeate samples was performed using a colourmetric method (Gallagher and Danielson, 1995). This is based on the reaction of a ferric ion (Fe^{3+}) with the lactone ring of the macrolide antibiotics. This method was

adapted by drying the clarified broth samples under vacuum for 2 h (Speed Vac SC 110, Savant, Farmingdale, NY) to avoid the interference of water on the assay. After drying, the samples were dehydrated by resuspending the pellet in glacial ethanoic acid to the original volume and heating at 45°C for 45 min. An equal volume of the assay mix, (320 mg iron III chloride in 8 mL water, 8 mL concentrated sulphuric acid and made up to 400 mL with glacial ethanoic acid) was added and this mixture was heated at 50°C for 15 min. The samples were then spun down at 13,000 rpm for 5 min to remove solids and the absorbance was measured at 592 nm. The erythromycin concentration in broth samples was subsequently determined from a standard curve prepared earlier.

Determination of Broth Viscosity

Broth viscosity was measured using a Contraves Rheomat 115 (Contraves Industrial Products Ltd., Middlesex, UK) at 20°C (Haake DC1 Circulator and K15 Bath; Haake, Sussex, UK). The viscosity of the SCM fermentation broth was measured using a concentric cylinder over a range of shear rates between 24.3 s^{-1} and 1256 s^{-1}. For the more viscous OBM broth a cup and bob attachment was used, because solids in the broth caused inaccurate readings due to friction between the particulates and the walls of the cylinders, this was carried out over a range of 6.65 s^{-1} to 117.1 s^{-1}. Both media were found to exhibit non-Newtonian behavior and both were found to adhere to the pseudoplastic Power Law or "shear-thinning" model of viscosity (Atkinson and Mavituna, 1991):

$$\tau = K\gamma^n \tag{1}$$

where τ is the shear stress exerted on the system, γ is the shear rate and K and n are the consistency and flow behavior indicies, respectively, that describe the behavior of the fluid. From the values of K and n, the apparent viscosity (μ_a) can be calculated:

$$\mu_a = K\gamma^{n-1} \tag{2}$$

The viscosity at the membrane surface is calculated by determining the shear at the membrane surface which is given by:

$$\gamma = \frac{6U}{b} \tag{3}$$

from Porter (1972), where U is the crossflow velocity and b is the channel height.

Determination of Residual-Oil Concentration

Residual-oil levels were determined using a hexane extraction based on the method by Junker et al. (1998). A 5-mL volume of sample was mixed with 15 mL of hexane for 3 min and centrifuged at 4000 rpm for 30 min. A 1-mL sample of the hexane phase was then dispensed into a preweighed aluminium container and dried at 85°C in an HG53 Halogen Moisture Analyzer (Mettler-Toledo, Leicester, UK), to constant weight. All samples were measured in duplicate and the mean and standard deviation calculated.

Glucose Assay

The glucose assay is based on the reducing sugar method with Dinitrosalicylic acid (DNS) as described by Miller (1959). Samples were diluted by factors of 10 and 20 with RO water, 0.4 mL of diluted sample were then dispensed into test tubes to which 0.5 mL of DNS reagent was added. Samples were incubated in boiling water for 5 min. Tubes were placed on ice and 4 mL of water added, absorbance was then read at 540 nm. Glucose concentrations were subsequently calculated from a standard curve.

Biomass Measurement

For the soluble medium biomass concentrations were determined by filtering a 5-mL broth sample through a 0.2 μm, 25-mm diameter filter (Millipore, Watford, UK). The biomass sample was then dried to constant weight using a HG53 Halogen Moisture Analyzer (Mettler-Toledo, Leicester, UK). All samples were measured in triplicate and a mean and standard deviation calculated. The biomass concentration of the OBM broth could not be determined due to the presence of undissolved soya flour.

Image Analysis

Image analysis was carried out using the method described by Packer and Thomas (1990). The image was processed by a Magiscan 2A Image Analyzer (Joyce Loebl Ltd., Gateshead, UK), which was connected to a Polyvar microscope (Reichert-Jung, Vienna, Austria) with video camera for image capture. The variables measured were main hyphal length (ML), branch length (BL), total hyphal length (HL), number of tips (NT), major axis (OL), minor axis (OB), mycelial area (MA), clump area (CA), projected area (OD), total area (OA) and circularity (OC) as described by Heydarian (1998). The number of objects measured varied from sample to sample however, the range was 223 to 941 individual objects.

RESULTS AND DISCUSSION

Fermentation Characteristics of SCM and OBM

The initial aim of the study was to compare the growth and antibiotic production rates of the two different types of media used. Figure 2 shows that there are a number of notable effects related to the medium composition. For the SCM fermentations the biomass concentration, reached a maximum about 35 h after inoculation, and the erythromycin A concentration reached a peak at approximately 70 h, where

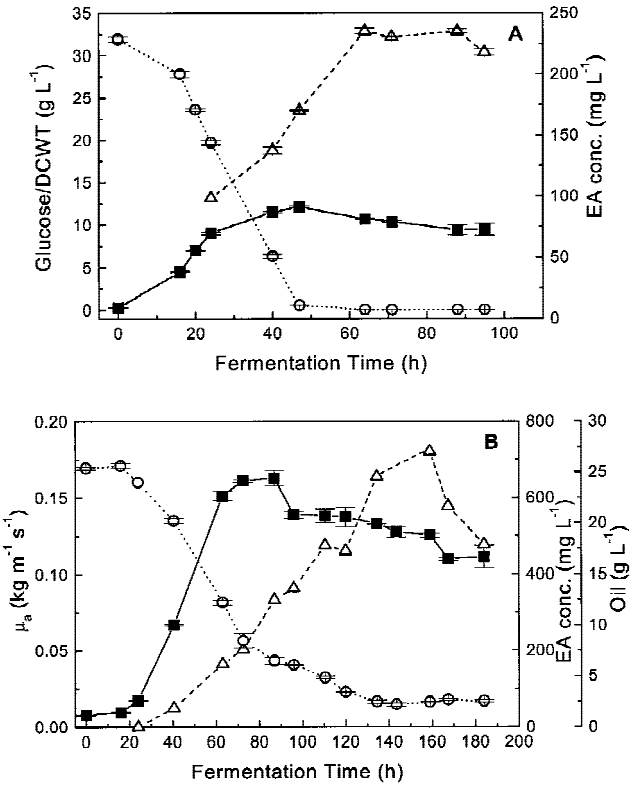

Figure 2. Examples of batch fermentation with *S. erythraea* CA340. (A) in soluble complex medium (SCM). Dry cell weight (DCWT: ■), residual glucose (○) and erythromycin A concentrations (EA: △) are shown. (B) in oil-based medium (OBM). Apparent viscosity at 71 s^{-1} shear rate (■), residual oil (○), and erythromycin A concentrations (EA: △) are shown. The error bars indicate deviations of duplicate measurements.

the concentration was 230 mg L^{-1}. The duration of the OBM runs were found to be significantly longer, because the volumetric rate of oil utilization (the main carbon source) was slower than the volumetic rate of glucose utilization in the SCM fermentations (0.29 gL^{-1} h^{-1} and 0.85 gL^{-1} h^{-1}, respectively, during exponential-growth phase). Volumetric rates of carbon source utilization have been expressed instead of specific rates because it was not possible to determine biomass concentrations in the OBM due to the presence of the undissolved soya flour. The presence of insoluble components in the OBM means that the biomass concentration cannot be measured directly. It has, however, previously been shown that an increase in viscosity can be taken as an indicator of increased biomass concentration (Karsheva et al., 1997). The viscosity of the OBM fermentation reaches a maximum at around 70 h, with the erythromycin concentration peaking at 160 h. The maximum titer of erythromycin A was 700 mg L^{-1}, which is approximately 3 times higher than for the SCM medium. Oil-based media are commonly used in antibiotic fermentations as they generally lead to high productivities and are inexpensive carbon sources (Zhang and Greasham, 1999).

The media composition also changed throughout the period of the fermentation as media components were utilized and biomass growth took place. Residual glucose and oil concentrations are also shown in Figure 2. The carbon source in the SCM is glucose, a small water-soluble molecule that will have little effect on the subsequent microfiltration of the broth. In contrast, oil is a known foulant of microfiltration membranes (Conrad and Lee, 1998) and because there may be residual oil left at the desired harvest time of the fermentation, (approximately 2.5 gL^{-1} at the maximum erythromycin concentration), it will have a negative effect on the permeate flux and may also influence antibiotic transmission. The OBM also contains another insoluble component, soya flour. Approximately 25% dissolves in the medium (figure quoted by supplier) leaving a considerable amount left in suspension. Although the amount of undissolved flour remaining at the end of the fermentation is not known because of the inability to separate it from the biomass, visual observation (increased packed-cell volume) and final total-solids concentration of the broth indicated that there was a significant amount left in suspension.

The on-line exit gas profiles are shown in Figure 3 for

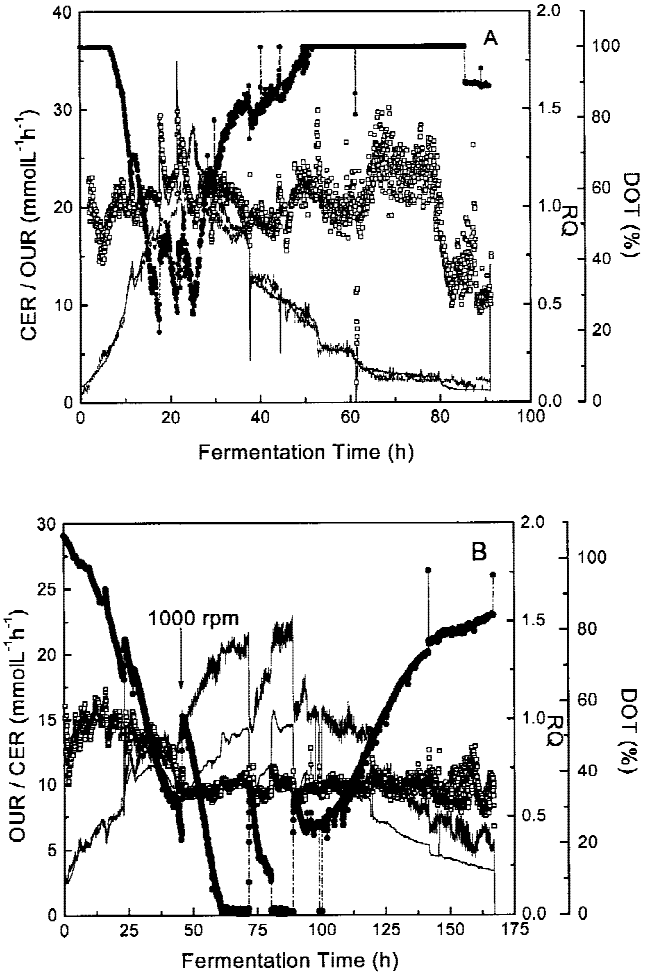

Figure 3. On-line exit gas data for two fermentations. (A) Soluble-complex medium (SCM), and (B) oil-based medium (OBM). — Carbon dioxide evolution rate (CER), - - - oxygen-uptake rate (OUR), -□- respiratory quotient (RQ), and -.•.- dissolved oxygen tension (DOT).

both SCM and OBM fermentations; carbon dioxide-evolution rate (CER), oxygen-uptake rate (OUR), the respiratory quotient (RQ) and the dissolved-oxygen tension (DOT) are displayed. From the RQ values it is clear that for the SCM the RQ is approximately 1, which is expected from growth on carbohydrates. For the OBM the organism initially displays an RQ of 1 as it uses the dextrin as the carbon source. When this is depleted the rape seed oil is used, and the RQ drops to around 0.7. The slower growth on oil is apparent because for the SCM fermentation the OUR and CER rise quickly and reach a peak at 35 h, corresponding to the maximum biomass concentration measured (see Fig. 2A).

The OBM broth, however, reaches a peak at 90 h and the OUR and CER increase relatively slowly. The viscosities of the two types of fermentation broth are also quite different. Although in both cases the broths adhere to a pseudoplastic rheology, the apparent viscosity of the OBM is approximately 2–3 times higher compared to the SCM values at the shear rates experienced at the membrane surface. The higher viscosity of the OBM is probably responsible for the lower DOT values recorded, compared to the SCM fermentations, due to a reduction in the oxygen combined mass-transfer coefficient K_La, (the rate of oxygen diffusion through the liquid films surrounding the gas bubbles would be proportional to $1/\mu$). This poor mixing around the DOT probe (as indicated by zero values of DOT and visual observation) can be improved with an increase in impeller speed, however, there is a maximum that can be achieved before the shear rate near the impeller will cause breakage of the mycelia (Heydarian, 1998). The apparent viscosity at a given shear rate can be calculated using the data obtained from the rheometer as described in the Materials and Methods section.

The apparent broth viscosity at a given shear rate can be calculated using data obtained from the rheometer (data not shown) and appropriate rheological models. Figure 4 shows the apparent viscosities of the two types of fermentation broths at the shear rate experienced at the membrane surface

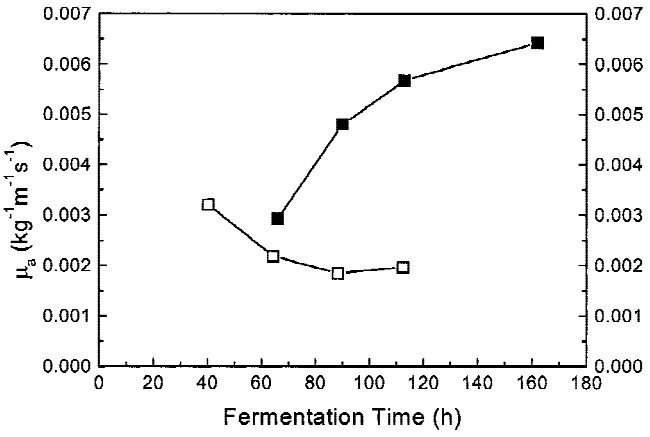

Figure 4. Variation of broth viscosity with fermentation time for soluble-complex medium (□) and oil-based medium (■). Apparent viscosity calculated at a shear rate of 11056 s^{-1} equivalent to that experienced at the membrane surface.

(11056 s^{-1}), i.e., the shear rate experienced by the broth during processing, as opposed to the values shown in Figure 2 which are measured at an mid-range shear rate from the viscometer (71 s^{-1}). It is clear that for the SCM, the apparent viscosity of the broth in the membrane module will decrease as a function of fermentation time, while that for the OBM broth shows a marked increase. It is difficult to specify an exact reason for this difference between the two media in such complex systems, but it could be due to a number of factors such as different biomass concentrations or morphologies, differing amounts of cell lysis or differences in the compositions of media components. The higher feed viscosities and solids-loading of the OBM broths is likely to have adverse effects on the microfiltration performance. The higher antibiotic titers reached, however, will increase the mass flux of erythromycin through the membrane. The relative magnitude of these two effects are discussed in more detail below. Permeate viscosities were also measured and found to be the same for both media. Both exhibited Newtonian characteristics which had viscosities approximately the same as water.

Microfiltration Characteristics of SCM and OBM Broths

The initial aim of the microfiltration studies was to examine the differences in filtration performance of the SCM and OBM broths with regard to the variation of permeate flux and erythromycin transmission as a function of transmembrane pressure. As can be seen in Figure 5, the permeate flux decays quickly over time for both types of broth as the membrane becomes fouled. The profile of the permeate-flux decline varies considerably between the two types of media which suggests differences in cake composition and deposition. In particular, the reduction in permeate flux follows a different time profile towards the establishment of steady state. The flux for the OBM approaches the steady-state flux more quickly than that of the SBM which indicates more rapid fouling of the membrane by the soya flour and oil-containing medium. A plot of the reciprocal of flux against microfiltration time is known to demonstrate the way in which the membrane is fouling (Tracy and Davis, 1994). These authors showed that in a model medium of yeast cells and BSA, internal blocking of the pores was characterized by a concave slope (the gradient of the slope increases with increasing time). Surface blocking of the membrane, on the other hand, would be characterized by a convex slope. For both types of medium used in this work, the slopes of the reciprocal of permeate flux against fermentation time were convex throughout the microfiltration time suggesting that the fouling is caused mostly by surface occlusion of the pores. A more-detailed discussion of the fouling mechanisms proposed for crossflow filtration can be found in Jonsson and Tragardh (1990).

Figure 6 shows the variation of steady-state flux with transmembrane pressure and the critical transmembrane pressure (cTMP), for each type of medium. It has been

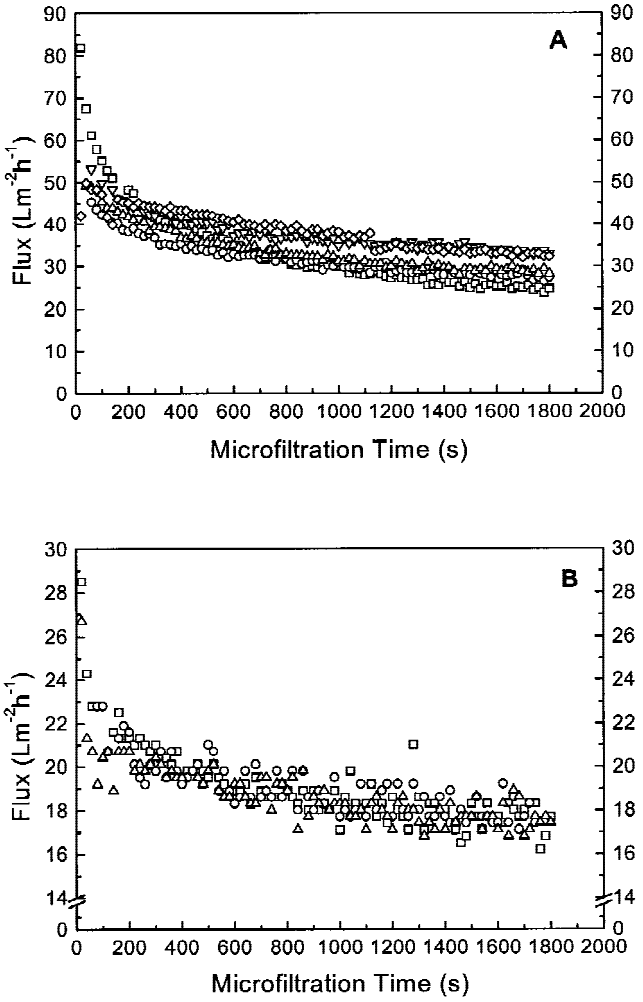

Figure 5. Variation of permeate flux over time at different transmembrane pressures. (A) For the soluble-complex medium, □: 12.5 kPa, ○: 22.5 kPa, △: 39.0 kPa, ▽: 56.5 kPa, ◇: 76.0 kPa for a broth sample taken at 88 h; (B) for the oil-based medium, □: 24.5 kPa, ○: 49.0 kPa, △: 75.0 kPa for a broth sample taken at 112 h, at constant crossflow 0.66 ms^{-1}.

previously described by Porter (1972) that compression of the cake occurs by increasing the transmembrane pressure, which leads to a decrease in the permeate flux. This can be seen in Figure 6 for the SCM broth which reaches a maximum and then decreases. It can be seen that for the SCM broth the value of cTMP is high at around 60 kPa, while for the OBM broth cTMP was much lower at around 22 kPa. In the latter instance, cTMP determination is limited by the minimum pressure that is imposed on the microfiltration system by the high broth viscosity, (the steady-state permeate flux did not increase after additional pressure was imposed by the backpressure valve). In the case of the OBM broth, the cake on the membrane surface has already formed and is being put under sufficient pressure at this point so as to become compressed.

To further understand the different flux profiles for the two media shown in Figure 5, the effects of different media components from the OBM, either individually or in combination, were examined in the absence of biomass. Rape

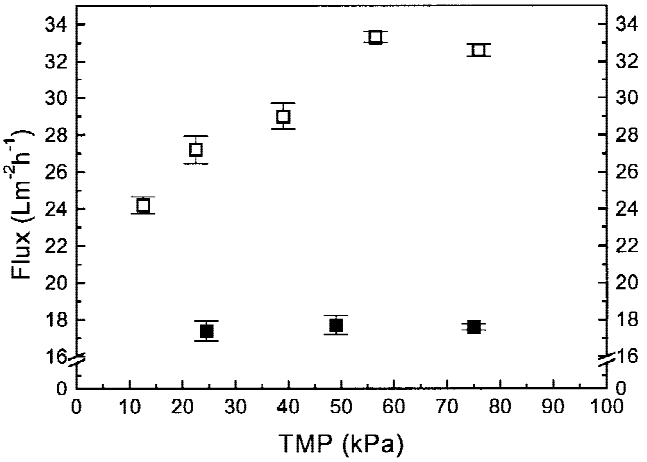

Figure 6. Variation of steady-state permeate flux with transmembrane pressure (TMP). □: Soluble-complex medium (SCM), ■: oil-based medium (OBM).

seed oil, soya flour, and the antifoam agent, PPG, were selected at representative concentrations likely to be found toward the end of the fermentation. The results are summarized in Table II, which clearly shows that the steady-state permeate flux in a simulated system appears to be dominated by the residual undissolved soya flour. The measured flux levels were approximately constant for all runs containing either flour on its own (~ 29 Lm^{-2} h^{-1}) or in combination with any of the other two media components (~ 30 Lm^{-2} h^{-1}). The cause of this limitation in permeate flux in the presence of soya flour is thought to be due to formation of a solid cake at the membrane surface rather than differences in permeate viscosity, this is supported by the measurements of the permeate viscosities of both media which were found to be similar. The measured permeate fluxes are, however, still around 20 Lm^{-2} h^{-1} higher than the whole broth samples (see Fig. 7), indicating a more complex interaction between the soluble and insoluble medium components in the whole OBM broth samples compared to the model systems used in Table II.

Variation of Microfiltration Performance with Fermentation Time

In each case, a broth sample was harvested from the fermentor at the end of exponential-growth phase and then

Table II. Measured steady-state permeate flux using various combinations of insoluble media components in water at 80 kPa TMP. X Indicates components present in a continuous-water phase.

PPG 0.2% (v/v)	RSO 0.3% (v/v)	Soya flour 1.0% (w/v)	Permeate flux (Lm^{-2} h^{-1})
X			953 ± 6
	X		251 ± 9
		X	28.3 ± 0.8
X	X		119 ± 2
X		X	30.8 ± 1.5
	X	X	31.2 ± 1.0
X	X	X	31.6 ± 0.7

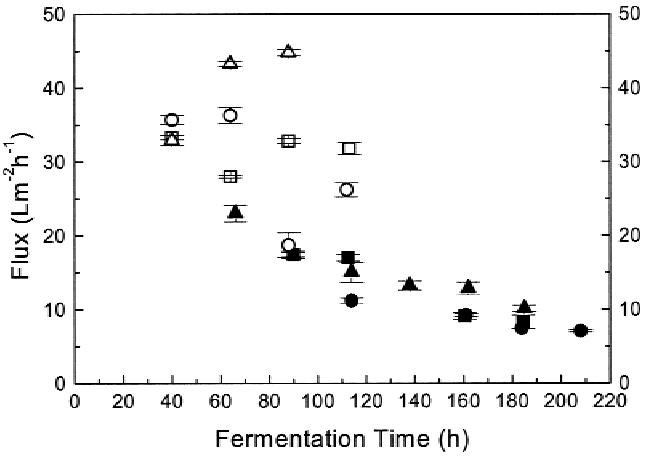

Figure 7. Variation of steady-state permeate flux against fermentation time for the six fermentations. □: SCM 1, ○: SCM 2, △: SCM 3, ■: OBM 1, ●: OBM 2, ▲: OBM 3.

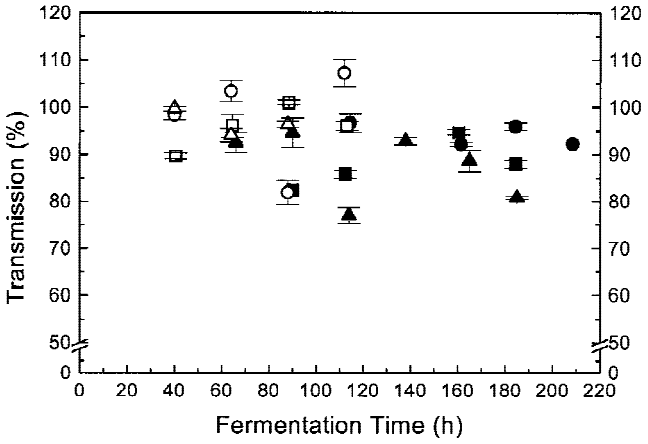

Figure 8. Variation of steady-state erythromycin transmission against fermentation time for the six fermentations. Transmission values were measured at the critical transmembrane pressure. □: SCM 1, ○: SCM 2, △: SCM 3, ■: OBM 1, ●: OBM 2, ▲: OBM 3.

every 24 h thereafter until the end of the fermentation. As previously shown in Figure 2, the composition of the two broths varies significantly over time, giving rise to different biomass, erythromycin, oil, and flour concentrations. This is likely to have a profound effect on microfiltration performance with regard to both permeate flux and antibiotic transmission. An understanding of this interaction between the fermentation and microfiltration processes will determine the optimal time to harvest the fermentor to maximize erythromycin recovery through the membrane and minimize processing time.

Figure 7 shows the variation of steady-state permeate flux for both SCM and OBM broth samples removed and processed at various times during the course of the respective fermentations (each performed in triplicate). It is apparent from Figure 7 that the steady-state permeate flux over the time course of each fermentation is more stable and reproducible for the OBM broth than for the soluble-complex medium. For the OBM broth, the permeate flux decreases asymptotically to the time axis for each fermentation, while for the SCM there is no obvious trend that can be drawn from the data. It is thought that this difference is due to the fouling of the membrane in each case. In the OBM fermentations, the limiting factor affecting flux is thought to be the media components and in particular, the residual flour (see Table II). In the SCM fermentations, however, the fouling layer will consist primarily of the biomass and the contents of lysed cells. This effect for the SCM broth was studied further using image analysis and is discussed in more detail in the next section.

In addition to a high-permeate flux being desirable, a high percentage of antibiotic transmission through the membrane (and fouling layer) is important in determining the overall product recovery. Figure 8 shows the percentage transmission of erythromycin across the membrane during the time course of the fermentation for both the SCM and OBM broths. The values displayed are those measured at the critical transmembrane pressure, although it was found that TMP had no effect on the transmission percentage. Although there is variation within each data set, a significant difference between the means of the transmission data for each type of broth was found. The calculated means are 96.7% ± 6.8 (w/w) transmission and 89.6% ± 6.1 (w/w) transmission for SCM and OBM broths respectively (p = 0.01116, two-population independent t-test). Erythromycin A (and its homologues) is a small molecule (ca. 730 Da) and therefore, should not be retarded by the membrane itself. Erythromycin may, however, become associated with the suspended solids in the medium (or filter cake) and may also dissolve in the residual oil of the OBM broth. Experiments to assess the levels of erythromycin adsorption onto the *S. erythraea* biomass and the solubility of the target molecule in the oil-phase, have shown that the amount of erythromycin lost from the continuous aqueous phase to these physical processes is insignificant. The equilibrium partition coefficient for erythromycin, K_{eryth} in a rape seed oil–water two-phase system (V_r = 1) was found to be 0.02, and this did not vary significantly over a pH range of 4 to 10, (the pK_a of erythromycin A is ≈ 8.6). The low loss of erythromycin to biomass adsorption and solubility processes is reflected by the fact that there is no significant variation in the transmission percentage across the membrane with fermentation time, over which the broth components are varying in concentration.

The mass flux of erythromycin across the membrane is of prime importance because this determines the duration of the microfiltration operation. Statistical analysis of the mass-flux data calculated from the steady-state permeate flux, transmission percentage, and concentration of erythromycin, showed that there was no significant difference between the means of the SCM and OBM broths, $SCM_{mean} = 1.47 \times 10^{-6}$ kgm^{-2} s^{-1}, $OBM_{mean} = 1.38 \times 10^{-6}$ kgm^{-2} s^{-1}. The low-permeate flux and slightly reduced erythromycin transmission observed in the microfiltration of the OBM broth is thus counteracted by the higher erythromycin titers

obtained during the fermentation. The choice between use of a soluble or oil-based medium would thus be based on economic criteria and the acceptable level of diafiltration.

Relationship Between Permeate Flux, Broth Viscosity, and Hyphal Morphology

The characterization of mycelial morphology using image analysis has been well documented. Equipment and techniques have been recently reviewed by Paul and Thomas (1998). Image analysis has been used previously to study filamentous organisms in liquid culture and observe how their morphology changes with time (Martin and Bushell, 1996; Treskatis et al., 1997). The effect of fermentor-operating conditions (type and operation) on *S. erythraea* morphology and erythromycin production has also been studied, (Bushell et al., 1997; Heydarian, 1998; Sarra et al., 1996). This technique has also been used to investigate how shape and species of single-celled bacteria affect the permeate flux in crossflow filtration (Nakanishi et al., 1987). A preliminary study was carried out here to determine if the cellular morphology of *S. erythraea* affected permeate flux and erythromycin transmission in both media. The aim of this work was to discover if changes in morphology over the course of the fermentation affected the microfiltration performance. Previous work by Sarra et al. (1996), using the same organism grown on a similar medium to our SCM, showed an increase in main hyphal length (ML) with fermentation time up to 35 h followed by a gradual decline.

Eleven image-analysis criteria for describing morphology were investigated as defined by Heydarian (1998). These were compared against permeate flux (J), transmembrane pressure (TMP), viscosity (μ, a combination of these factors; resistance to flux (R_t), as given by Equation (4) and erythromycin transmission (ϕ).

$$R_t = \frac{TMP}{J\mu} \tag{4}$$

As shown in Figure 9 there appeared to be a linear, inversely proportional relationship between the major hyphal length of the mycelia and the steady-state permeate flux. The major hyphal length is described as the length of the longest hypha in the mycelium. For SCM 1, $p = 0.0019$, $R^2 = 99.99\%$ and for SCM 3 $p = 0.0488$, $R^2 = 98.83\%$. Image-analysis data were not available for SCM 2. This preliminary observation, although not conclusive, does indicate that it is the morphology of the cells that determines microfiltration flux in the soluble medium. Such an effect has previously been shown in nonfilamentous organisms (Nakanishi et al., 1987), where size and shape between several species has had an effect on filtration performance. It was found that rod-shaped bacteria formed a cake with 100 times higher resistance than that formed by elliptical bacteria and showed a relationship between the compressibility and the void fraction of the cake. Within a single species of unicellular organisms the main effect has been shown to be the dry cell

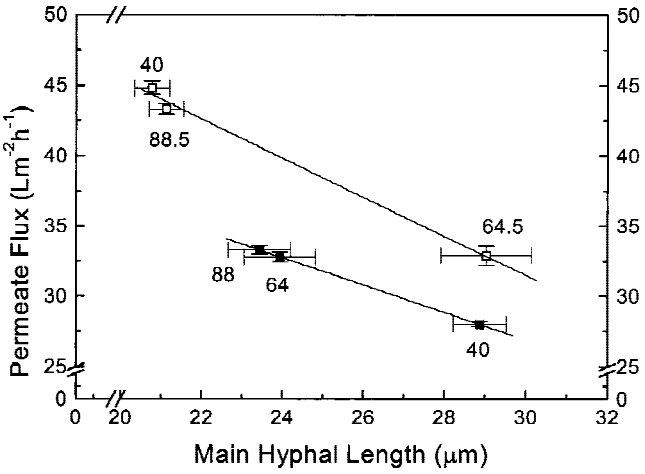

Figure 9. Correlation of mean main length of mycelia (ML) against steady-state permeate flux for duplicate fermentations of soluble complex medium. □: SCM 1, ○: SCM 2. The numbers represent the time in hours after inoculation when the samples were taken.

weight (Meyer et al., 1998). In the case of *S. erythraea*, as used here in the SCM broth, permeate flux was found to be independent of the fermentation time (Fig. 7), the viscosity, and dry cell weight, but the morphology is apparently a significant factor. The hyphal morphology may vary due to a number of biological factors (e.g., nutrient limitation) and/or engineering parameters such as power input (Heydarian, 1998).

No relationship between hyphal morphology and steady-state permeate flux was observed in the OBM fermentations, which tends to confirm that in this case, the media components are the determining factors for the permeate flux rather than the biomass. This hypothesis is partly supported by work carried out by Hong et al. (1997), where permeate flux was dependent on particle size in a colloidal suspension of silica (SiO_2). For a soya flour suspension, particle size shows a normal distribution around a maximum of 100 μm (data from supplier). It was also reported by Hong et al. that in their SiO_2 suspension the permeate flux was independent of crossflow velocity under laminar flow conditions.

CONCLUSIONS

It is clear that fermentations and the processes downstream of them cannot be considered as separate entities. It has been demonstrated in this study that changing the fermentation medium to increase product titers has been shown to adversely affect microfiltration performance. The drop in steady state permeate flux and decreased erythromycin transmission seen by using an oil-based medium mean that despite increased feed stream concentration of antibiotic, the mass flux through the membrane is not improved. The cost of the medium however, particularly for large scale industrial fermentations, will have been considerably reduced. The results of this work also suggested that micro-

filtration of simulated broths may not give a clear picture as to how an actual broth will affect the performance of a microfiltration operation. The dependence of steady state permeate flux on the morphology of the organism, in soluble complex media and the ability to predict the morphology from fermentation power input (Heydarian, 1998) needs further study. This work is currently underway in our laboratory with a view to being able to predict microfiltration performance from fermenter operating conditions.

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NOMENCLATURE

b	is the channel height of the flow path through the crossflow-filtration device (m)
BSA	bovine serum albumin
CER	carbon dioxide evolution rate (mmol $L^{-1}h^{-1}$)
DCWT	dry cell weight (g L^{-1})
DOT	dissolved oxygen tension (%)
EA	Erythromycin A
HPLC	high-pressure liquid chromatography
J	flux of the permeate ($Lm^{-2}h^{-1}$)
K	consistency index (dimensionless)
K_{eryth}	erythromycin partition coefficient (concentration in oil/concentration in aqueous solution (dimensionless)
K_La	Mass transfer coefficient (s^{-1})
ML	main hyphal length (μm)
N	flow-behavior index (dimentionless)
OBM	oil-based medium
OUR	oxygen uptake rate (mmol $L^{-1}h^{-1}$)
p	probability significance level (dimensionless)
PPG	poly-propylene glycol (antifoam)
R^2	the square of the Pearson product moment correlation coefficient (dimensionless)
R_t	total hydrodynamic resistance (s)
RO	reverse-osmosis water
RQ	respiratory quotient (= OUR/CER) (dimensionless)
RSO	rape seed oil
SCM	soluble complex medium
TMP	transmembrane pressure (Pa)
cTMP	critical transmembrane pressure (Pa)
U	crossflow velocity (ms^{-1})
V_f	phase volume ratio (= V_{oil}/V_{aq}) (dimensionless)
V_r	Volume ratio (volume of oil/volume of aqueous phase) (dimensionless)
vvm	volume of air per volume of medium per minute (min^{-1})
ϕ	erythromycin transmission (%)
γ	shear rate (s^{-1})
τ	shear stress (Pa)
μ	viscosity of the liquid ($kgm^{-1}s^{-1}$)
μ_a	apparent viscosity ($kgm^{-1}s^{-1}$)

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