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IN SITU FILTRATION OF *ANCHUSA OFFICINALIS* CULTURE IN A CELL-RETENTION STIRRED TANK BIOREACTOR

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SUMMARY

The effect of agitation and aeration on filtration of *Anchusa officinalis* culture in a stirred tank bioreactor integrated with an internal filter unit was investigated. Increases in suction head of the pump that drove the filtration process were measured at impeller speeds of 100 and 200 rpm. Surprisingly, suction head attained at 200 rpm was about 40% higher than at 100 rpm. Direct observation of the cake deposition process in the reactor using a dilute cell suspension revealed that the filter cake formed at 100 rpm was thicker, but less compact. Aeration at 0.4 vvm was shown to have little effect on the filtration rate, since the bulk fluid flow was dominated by the impeller hydrodynamics. The initial flux can be recovered by filter backwashing with compressed air at a flow rate of 0.6 vvm for a duration of 5 minutes.

INTRODUCTION

Perfusion plant cell cultivation can improve the production of such secondary metabolites as berberine (Fujita, 1988) and rosmarinic acid (Su et al., 1993), but effective cell-retention bioreactors are required. These bioreactors are also useful in two-stage cultivation for secondary metabolite and somatic embryo production (Fujita, 1988; Ammirato and Styer, 1985), where spent growth medium needs to be replaced by the production medium at the end of the growth stage. Currently, cell/medium separation in cell-retention reactors is based on either sedimentation or filtration. In designing a cell-retention bioreactor for cultivating high density plant cell suspensions, in which PCV (packed cell volume) may exceed 60% (v/v), problems in circulating viscous and shear sensitive cell cultures through an external loop, where anoxic condition may occur (Holst and Mattiasson, 1991), make the use of external separation devices less attractive.

Previously (Su and Humphrey, 1991), effective medium exchange was demonstrated in *Anchusa officinalis* cultivation using internal filtration in a membrane-aerated stirred tank reactor which resembled the "filter fermenter" described by Dostálek and Häggstrom (1982). This reactor has been modified in the present study to improve mixing and oxygen transfer by replacing membrane-aeration with sparged-aeration, and using the filtration chamber as the reactor base (the filtration stirred tank reactor, FSTR, Fig. 1). The design and arrangement of the filtration chamber in the FSTR avoids many problems associated with other existing internal separation devices such as spin filters, internal microfiltration with hollow fibres, and internal settling columns. For spin filters, the centrifugal force generated via the rotation of the cylindrical filter is expected to hinder filter fouling. However, it has been shown by

Yabannavar et al (1992) that high spinning rates also promote fluid exchange across the filter, leading to a reduced particle retention efficiency. When spin filters are applied in suspension cultures of plant cells, filter fouling is particularly problematic because a thin layer of cells tends to coat on the outer surface of the filter (Su and Humphrey, 1991). Difficulty in achieving efficient backflushing in spin filters further limits their usefulness in plant cell cultures. The major problem for internal microfiltration with hollow fibers is that commercial hollow fibers have pore sizes much too small for plant cell suspensions, and hence filter fouling becomes inevitable. Also, the presence of the hollow fiber bundles in the reactor may disrupt the flow pattern. As for internal settling columns, cell sedimentation is reduced substantially at high biomass concentrations due to particle interactions, which prevents the use of high medium-exchange rates, and the long cell residence time in the settling column may jeopardize cell activities.

Since the filtration efficiency of the FSTR is expected to strongly associate with the hydrodynamics in the reactor, the present study was undertaken to investigate the effect of agitation and aeration on the filtration of plant cell culture in the FSTR. *A. officinalis* was used as the model cell culture.

MATERIALS AND METHODS

Plant Cell Culture

The stock *A. officinalis* culture was maintained in a liquid Gamborg B5 medium (Gamborg et al., 1968) supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 0.1 mg/L kinetin, and 30 g/L sucrose (De-Eknamkul and Ellis, 1984). The suspension was subcultured every 10 days using a 10% inoculum.

The Bioreactor

A 2.5 liter BioFlo IIc stirred tank fermenter (New Brunswick Scientific) was modified to incorporate a filtration chamber at the base (Fig. 1). The non-jacketed glass cylinder, used as the BioFlo IIc reactor body, was replaced by a jacketed cylinder. In addition, the original dished-bottom water jacket assembly was substituted by a specially designed filtration chamber. A stainless steel composite disc filter with 70 μm pore opening and an effective filter area of 44 cm^2 (Fuji Filter Manufacturing Co., Japan) was used as the filter medium. The reactor was equipped with dual Rushton turbines (impeller diameter = 6.5 cm) placed 1.5 impeller diameter apart along with four baffle plates and an extra-coarse sintered glass sparger (maximum pore size range = 145 ~ 174 μm) for agitation and aeration. The lower impeller was placed 3 cm above the filter disk.

Culture Filtration Experiments

A. officinalis culture at a known cell density was charged into the reactor operating at preset agitation and aeration rates. During the filtration flux measurement, cell concentration in the reactor was kept nearly constant (cell loss through the filtrate was negligible). The feeding pump, which was controlled through a level probe located in the reactor, was set at a speed slightly lower than the filtration pump. As the filtration flux gradually decreased with time, the liquid level contacted the level probe and the feed pump was temporarily turned off until the liquid level was below the level probe. Since the duration of each experiment was short (less than two hours), cell growth was insignificant. Foaming was controlled by adding 100 ppm antifoam (Antifoam C emulsion, Sigma Chemical). The flux was estimated from the rate of increase in the accumulated filtrate volume measured using an electronic balance. For those experiments where only pump suction head was measured, feed pump was turned off, the level probe was removed and the filtrate was recycled back into the reactor.

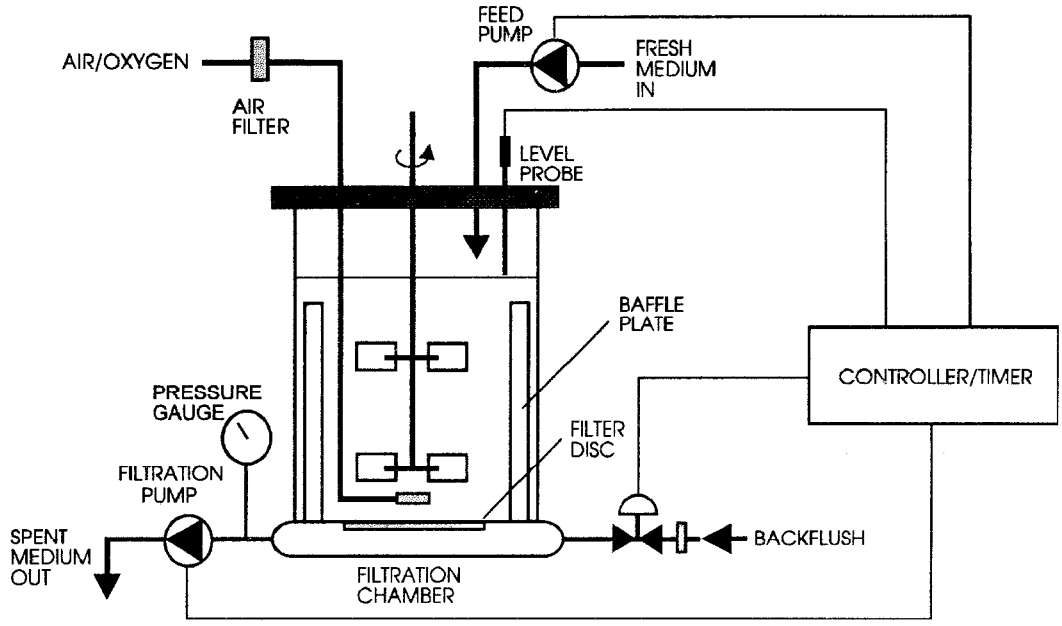

Fig. 1. Reactor set-up for the culture filtration experiments.

RESULTS AND DISCUSSION

The pressure drop that drives the filtration process in FSTR is provided by means of a suction pump located down stream of the filter (Fig. 1). During the filtration process, flux is related to the pump suction head according to the pump characteristics. As the filter gradually gets clogged, the pump suction head increases and the flux decreases. The concept of using a rotating scraper unit placed immediately above the filter medium to reduce the filter cake thickness has been employed in many filtration devices. For example, Tiller and Cheng (1977) in their study of so-called "delayed cake filtration" for the filtration of clay slurry, the cake thickness was found equivalent to the clearance between the scraper and the filter (3 mm in this case) at low agitation speeds, while under high agitation speeds, liquid shear was high enough to decrease the cake thickness. A similar concept has been applied in FSTR, where the impeller sits directly above the filter disk. Of course, the situation is more complex in this case, since the impeller also serves as a mixing and mass transfer device. To examine the impact of agitation on the filtration flux in FSTR, increases in pump suction head during filtration were measured at 100 and 200 rpm, with (0.4 vvm) or without aeration. Agitation rates above 200 rpm were not tested due to possible shear damage on the *Anchusa* cells (Lei, 1994). The result of the filtration experiment without aeration is shown in Fig. 2. The cell concentration in the reactor was 35 g dry weight/L (PCV = 80%). Pump speed was set at a constant level that gave a water flux of 1.2 $cm^3/cm^2 \cdot min$. Surprisingly, a higher suction head was attained at 200 rpm than at 100 rpm. Immediately after the filtration was initiated, a very sharp increase in the suction head was observed, which was followed by a more gradual increase. During the early stages of

filtration in FSTR, filtrate flux was high relative to the fluid flow tangential to the filter that was caused by mixing. Consequently, the flux dominated the fluid transport process and cell particles might be advected almost perpendicularly onto the filter medium. This is corresponding to the initial sharp increase in the suction head and the rapid cake buildup. As the filtration process proceeded, the cake thickness increased and the flux decreased, the tangential fluid flow then became dominant. At this stage, the particle trajectories might be nearly parallel to the cake. According to Mackley and Sherman (1992) in their study of cross-flow cake filtration with 125-180 μm polyethylene particles, when the tangential fluid flow dominated, particles were seen to roll along the cake surface until captured at a stable site, and this packing process appeared to be very selective. Accordingly, a very compact cake with high specific cake resistance was formed in the presence of tangential flow. The effect of particle packing selectivity was found to be more profound for the larger particles than the smaller ones. For instance, for calcite of mean particle size 27.5 μm, an increase in cross-flow velocity led to a decrease in filtrate flux whereas for a mean size of 2.7 μm, the opposite occurred (Wakeman and Tarleton, 1991). Cultured plant cells are quite large in size (10 to 100 μm in diameter), and more so they are usually present as cell aggregates. Therefore, the cross-flow dependent cake packing should be a real concern. This was confirmed by visualization of the cake formation in FSTR using a dilute cell suspension (cell density = 1 g dry weight/L). Under low agitation rates (less than 100 rpm), the filtrate flow was higher than the lifting bulk fluid flow. As a result, a thick cake was formed immediately. This cake, however, was very fluffy and could be readily resuspended into the culture by agitation after the suction pump was turned off. As the agitation rate increased, the cakes were present as small, unevenly distributed thin patches. These thin cakes appeared to be very compact. They could only be resuspended back to the culture by filter backflushing.

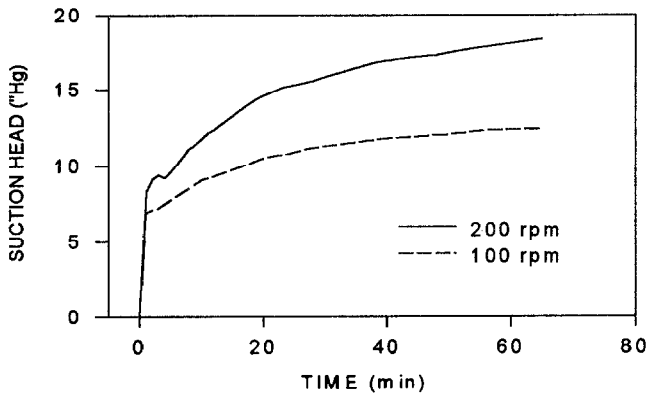

Fig. 2 Increase in the pump suction head during the culture filtration experiment in FSTR.

When the culture was aerated at 0.4 vvm, similar pressure profiles to those shown in Fig. 2 were observed (data not shown). At this aeration rate, the bulk fluid flow was still dominated by the hydrodynamics of the impeller, and therefore a similar suction head profile was observed as in the nonaerated culture. As the aeration is increased, especially at higher agitation rates, large gas-filled cavities behind the impeller blades may appear leading to a lower pumping capacity of the impeller (Nienow and Ulbrecht, 1985). This condition may reduce the filter fouling compared with lower aeration under the same agitation rate. Nevertheless, since this condition is not favorable from a mixing perspective, and high agitation and aeration are unlikely to be used in plant cell cultures, aeration should have little effect on the filtration process in FSTR, as long as the bulk fluid flow is dominated by the hydrodynamics of the impeller.

Flux decay due to increased mass transport resistances is inevitable in any filtration process. It is a general practice to use backflushing to clean the filter. The success of backflushing depends on the flux decay mechanism which is determined mostly by the characteristics of the filter and the cell culture. In some cases, for instance during the cultivation of slime-forming bacteria in the "filter fermenter", filter may be irreversibly blocked after only a short operating period leading to shutdown of the reactor (Dostálek and Häggstrom, 1982). Fig. 3 shows the effect of backflusing on the filtrate flux during the *in situ* filtration of *A. officinalis* culture in the FSTR. Periodic backflushing was done by passing compressed air into the filtration chamber at a flow rate of 1 liter/min, while the filtration was stopped. In one treatment, the filter was backwashed for 10 seconds following every 2 minutes of filtration, while in the other treatment, it was backwashed for 50 seconds for every 10 minutes of filtration. As shown in Fig. 3, the former treatment was more effectual in sustaining the flux. The initial flux can be recovered in both treatments, however, by backwashing at a higher air flow rate (1.5 l/min) and longer duration (5 minutes) (Fig. 3). On the basis of this result, a two-compartment filtration/backwash chamber is proposed (Fig. 4). While spent medium is removed through one of the compartments, the other compartment is purged with air for backwash and culture aeration. This process is alternated between the two compartments by controlling the on/off cycle of the solenoid pinch valves using a programmable timer. Testing of this design is currently underway.

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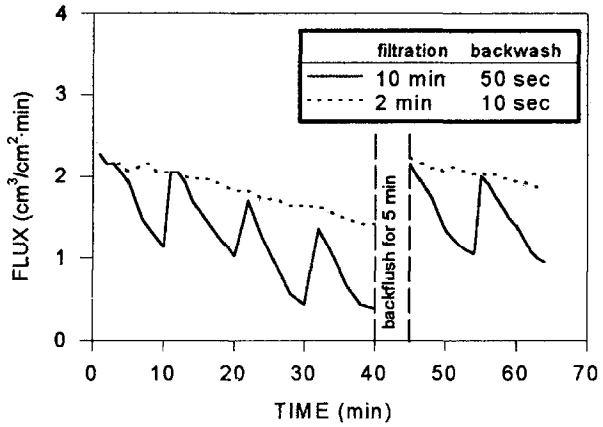

Fig. 3 Effect of backflushing on the filtration flux in FSTR. Cell density = 12.5 g dry weight/L, agitation = 100 rpm, aeration rate = 0.4 vvm.

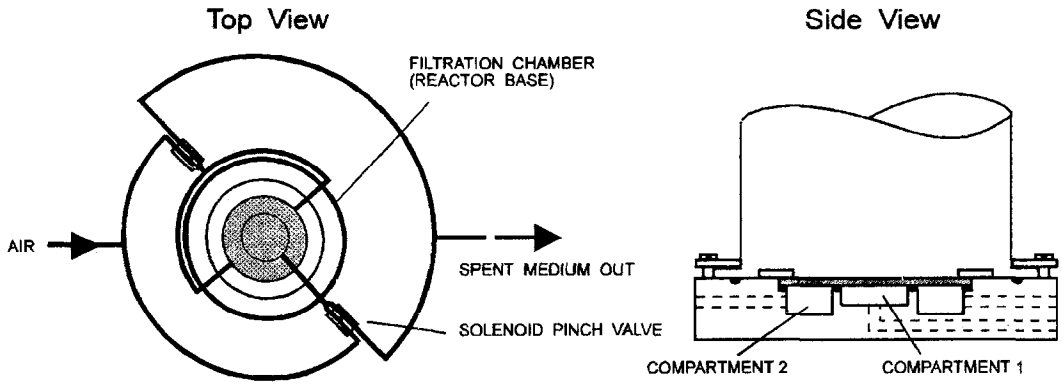

Fig. 4 The two-compartment filtration/backflush chamber for the FSTR.

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