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Solid-state fermentation and bioremediation: development of a continuous process for the production of fungal tannase

J. van de Lagemaat, D.L. Pyle*

Biotechnology and Biochemical Engineering, School of Food Biosciences, The University of Reading, P.O. Box 226, Whiteknights, Reading RG6 6AP, UK

Abstract

A solid-state fermentation (SSF) process for the continuous production of the enzyme tannase is currently being developed. This paper describes a laboratory scale prototype reactor that was built with the specific aim in operating continuously with solid substrates and without inoculation of the feed. Mixing, fungal growth and sporulation studies have been conducted as a basis for successful operation of a truly continuous process. The mixing studies at different feed rates revealed a mixing pattern with near perfect mixed flow and the solids mixing in the reactor have been characterised using a dispersion model. Lower biomass, tannase yield and sporulation rate were obtained in a rotating batch culture at 0.7 rpm compared to static batch culture, presumably due to the deleterious effect of shear. The results of this and future research will be used in order to predict operation variables in the continuous SSF process.

Keywords: SSF process; Tannase; Polyurethane foam

1. Introduction

Solid-state fermentation (SSF) has been defined as a process in which microorganisms are grown on solid substances in the absence or near-absence of free water [1]. With the widespread use of inert supports in which an excess of free water can be present the definition of Aidoo et al. [2] is more appropriate: an SSF process means any fermentation that takes place on a solid or semisolid substrate or that occurs in a nutritionally inert support. In general, SFF processes will take place under low water/high solids conditions. Microbial attachment to the solids, whether inert or degradable, and the low water conditions make SSF rather different from the more common submerged fermentation. From the point of view of process intensity a key question is to what extent the advantage of operating in a concentrated environment is offset, if at all, by changes in the process kinetics. If the process intensity and stability can be assured, SSF could be the system of choice for selected "added-value" applications such as the production of fermented foods, enzymes and other extracellular metabolites like organic acids and flavour compounds. Applications of SSF to other than purely profit-driven objectives, such as environmental control, include the production of compost and animal feed from solid waste [3]. Our research attempts to combine both the "added-value" and environmental control features of SSF by developing a continuous SSF process for the production of the scarce fungal enzyme tannase from the waste product coffee pulp whilst stabilising the coffee pulp. Tannase has many potential applications in the food, pharmaceutical and chemical industries but due to the shortage and high cost of the enzyme, the use of tannase in large-scale applications is limited at present. It is hoped therefore that the economic benefits of tannase production can help improve the overall viability of the process.

The research carried out here is part of a collaborative effort of research teams from France, the UK, Mexico and Brazil to recycle coffee pulp and coffee husk by biotechnological processes. Mexico and Brazil alone produce around 400,000 metric t of grain coffee each year and thereby generate great volumes of coffee by-products [4]. Coffee pulp comprises the skins and pulp surrounding the coffee bean and is produced in the first stage of coffee processing. Its high polysaccharide and moisture content (which depends on whether a "dry" or "wet" process is adopted) make the pulp highly degradable. Currently much pulp is dumped — the fermented solids are an embarrassment and the waters leaching from the fermenting piles are highly polluting. The presence of recalcitrant and toxic compounds such as caffeine, tannins and polyphenols limit the use of coffee pulp/husk as a feed source for animals [5] and can also cause

* Corresponding author. Tel: +44-118-9318700; fax: +44-118-9310080.
E-mail address: d.l.pyle@afnovell.reading.ac.uk (D.L. Pyle).

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serious environmental contamination. A multi-step SSF using a mixed inoculum of lactic acid bacteria and various filamentous fungi has been proposed to give a preserved and detoxified product that can be used for animal consumption, mushroom, fungal metabolites and enzyme production [4]. The development of the continuous SSF process described here has to become part of this overall solution.

To the best of our knowledge all current SSF processes such as composting, mushroom production, and the Koji fermentations are operated in batch mode. Disadvantages of this mode are the requirement for regular re-inoculation and the low productivity inherent in repeated batch operation. One of the objectives of this research, therefore is to develop a continuous SSF process. In the past, the term "continuous SSF" has been used for a constantly moving series of batch fermentations such as the conveyor-type fermenter or for discharge-fed batch systems [6]. A true continuous SSF system is defined here as a reactor with sufficient back mixing that it can operate with a constant sterile feed in a way comparable to a chemostat. The conditions in the system remain stable throughout the fermentation and there is a balance between the material leaving and entering the reactor and a balance between microbial cells leaving the reactor and growing in it. To our knowledge, a true continuous SSF has never been performed but Hrubant et al. [7] developed a process close to this. The substrate was tumbled in a three-chambered, baffled cylinder in which small amounts of animal waste and grain were introduced into one end of the vessel and product was collected at the other end of the reactor. The fermenter operated for 63 days but was fed continuously with waste containing microflora, which functioned as inoculum. In a truly continuous process, inoculum has to be added only once at start-up of the fermentation.

It is important to have sufficient back mixing within the reactor to enable operation with a sterile feed, i.e. the solid substrate in the vessel containing microbial cells or spores has to inoculate the sterile feed entering the reactor. The key aim of this work is to establish how to define "sufficient mixing" as a function of the fungal growth kinetics and the fermenter operating conditions, and to test these concepts experimentally. Mixing of the fermenting mass also has beneficial effects like improving homogeneity, prevention of clump formation, promotion of gas transfer and facilitation of heat exchange [8]. These advantages were the basis for agitated reactors such as the rotating drum fermenter [9] and a variety of reactors consisting of a stationary vessel in which there is a device to mix the fermenting mass [10]. Several workers have studied the effect of rotational mixing on mycelial damage but some of the reports are contradictory. High rotational speeds in rotating drum reactors often reduced SSF productivity or sporulation, probably due to the damaging effects of shear [9,11,12]. However, in a study of Lindenfelser and Ciegler [13] maximum productivity was achieved at the highest rotational speed.

The concept of the tannase production process is to use coffee pulp or coffee pulp juice as a tannin-rich substrate and achieve direct breakdown of the hydrolysable tannins present. A study by Lekha and Lonsane [14] and our own findings suggest that SSF is advantageous over conventional submerged fermentation for the productive yield of this particular fungal enzyme. The developed process could potentially be used with other tannin-rich agricultural residues such as cassava, carob bean, wine-grape and tea waste. More generally, a continuous SSF process has promising applications for the production of bioactive compounds, enzymes and organic acids from different agricultural waste products such as sweet potato residue, sugar beet pulp and carrot-processing waste. In the case of coffee wastes, since tannase is an inducible enzyme, fungal growth and tannase productivity on coffee pulp or coffee pulp juice are being studied as part of this project. Here we will report on studies of a model SSF system where, instead of coffee pulp, we use tannic acid as the principal carbon source.

2. Experimental

In this work, following extensive screening studies, the filamentous fungus *Penicillium glabrum* was chosen for study on the basis of its high productivity as a source of extracellular tannase. The enzyme produced by this particular strain can easily be purified since it is the only tannase as could be seen from the single activity band in enzyme characterisation studies [15]. The organism was cultivated on a model SSF system consisting of polyurethane foam (PUF) cubes impregnated with a liquid medium containing tannic acid as the main carbon source. Natural substrates such as coffee pulp have the major complication that the carbon source is part of the structure. The geometric and physical characteristics of the substrate change since the medium is degraded during microbial growth, thus limiting the direct determination of key variables such as fungal biomass. The use of the inert PUF as support can overcome this problem because it has a constant physical structure throughout the fermentation. Furthermore, it allows easy separation of the enzyme from the inert carrier and enables a controlled medium composition. Mass balances for process modelling and control are more easily established because all concentrations of the nutrients in the medium are known and can be analysed.

A laboratory scale prototype reactor was built with the specific aim of operating continuously with solid substrates and without inoculation of the feed. The design is an inclined, rotating, baffled cylinder with continuous feeding and sampling devices at both ends of the reactor. This paper will present studies on the fungal growth process, and mixing and modelling studies carried out in the reactor. We will compare the results of physiological studies in both static and rotating batch culture to elucidate fungal sporulation patterns, growth behaviour and enzyme production. This work will then briefly be discussed in the light of the aim to develop a true continuous fermentation with coffee pulp as the solid substrate.

3. Materials and methods

All chemicals used were analytical grade and purchased from Sigma (Poole, UK) unless otherwise stated.

3.1. Fermenter design

The reactor consisted of an inclined, rotating, baffled cylinder with a feeding device at the right-hand side of the cylinder and a sampling device on the left-hand side (Fig. 1). A Plexiglas tube (1 m × 0.08 m) was made of two half cylinders clamped together by three metal rings which could be opened for sampling. The two separate cylinders contained 10, 0.02 m wide baffles each, which formed a counter-clockwise spiral continuum when brought together. The tube was placed onto two lines of rollers connected to a motor that made it rotate counter-currently at speeds of 0.7–2.0 rpm. Due to the (variable) inclined angle of the tube, downward movement of the particles in the tube was achieved. At the same time, there was also an upward movement, causing back mixing, of the particles caused by the rotating spiral continuum. If the feed and exit ports were closed the angle could be adjusted to give a uniform solid hold-up throughout the length of the reactor. Batch fermentations were carried out in this mode. In continuous mode the solids hold-up in the reactor depended on the angle of inclination and the solids feed rate. The feeding device consisted of a plastic screw that could be filled with the PUF particles, i.e. the substrate, mounted in a metal tube. A sampling device made of a rotating disk with beakers was placed just below the exit opening of the tube. Both the feeding and sampling devices were connected to a second motor, which was able to achieve feed rates of 300–600 ml per day.

3.2. Microorganism

P. glabrum (Wehmer) Westling (FST 99) was obtained from the Food Biosciences culture collection of the University of Reading. Malt extract agar (Oxoid, Basingstoke, UK) slants were used for sporulation. Spore suspensions were prepared in 10 ml sterile 0.2% Tween 80 after an incubation period of 5 days at 30°C.

3.3. Inert carrier

PUF-type HR 40 was supplied by Foam and Fiber (Bracknell, UK) and had a bulk density of 40 kg/m^3. The foam was cut into 6 mm cubes, washed three times with warm water and dried for 4 days at 65°C. The foam was pre-weighed and transferred to 250 ml Erlenmeyer flasks. After sterilisation for 15 min at 121°C, the foam was dried at 65°C for 1 day.

3.4. Solid-state fermentations on PUF

A static batch fermentation was carried out in 250 ml Erlenmeyer flasks and a rotating batch fermentation was performed in the fermenter with the tube rotating horizontally at 0.7 rpm. The baffles in the tube were removed and 20 compartments were created with aluminium mesh disks. The tube was sterilised with disinfectant and both ends were closed up with cotton wool. The feeding and sampling devices of the fermenter were not used.

The liquid medium which was added to the PUF had the following composition (w/v): 5% tannic acid, 1.0% NH_4NO_3, 0.5% KH_2PO_4, 0.25% glucose, 0.1% $MgSO_4{\cdot}7H_2O$, 0.01% $CaCl_2$, 0.002% $MnCl_2{\cdot}4H_2O$, 0.001% $FeSO_4{\cdot}7H_2O$ and 0.001% $Na_2MoO_4{\cdot}2H_2O$ in 0.15 M phthalate buffer (pH 5.5). Tannic acid, glucose and mineral solutions were prepared separately. The pH of the tannic acid solution was adjusted to 5.5 with 1 M NaOH and the solution was sterilised with a 0.45 μm syringe filter (Gelman, Ann Arbor, USA). The other solutions were heat-sterilised for 15 min at 121°C. The solutions were added together and inoculated with 3.5×10^{10} spores/g tannic acid, 15 ml of the inoculated liquid medium was added to a 250 ml Erlenmeyer flask containing 0.7 g PUF and mixed thoroughly; the flask was closed with a cotton plug. The 0.7 g impregnated PUF for the rotating batch experiment was transferred to the corresponding compartment in the tube. Incubation took place at 30°C for 2–7 days and

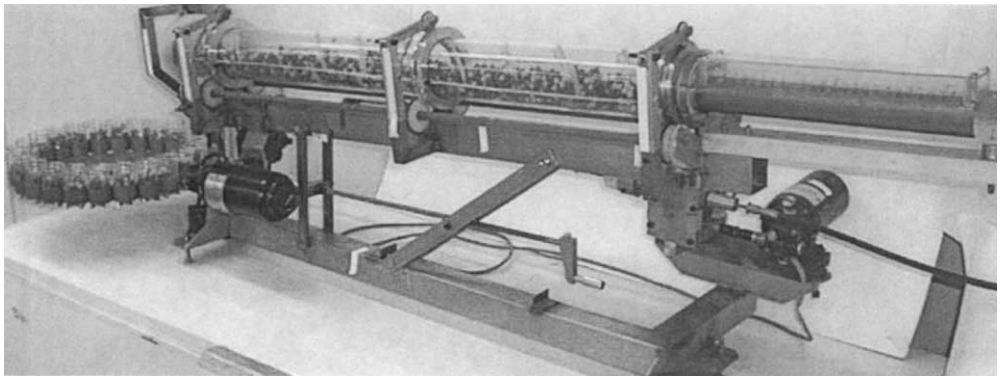

Fig. 1. Overall view of the continuous solid-state fermenter.

samples were taken every 6, 8 or 24 h depending on the type of experiment by removing a single Erlenmeyer flask from the incubator or by emptying a compartment. The fermentations were carried out in duplicate.

3.5. Sample treatment for biomass determination

The samples were weighed precisely directly after sampling. Following this, 10 ml of distilled water was added and mixed thoroughly with the PUF. This was placed into a Buchner funnel containing a 0.2 μm pre-weighed filter (Gelman, Ann Arbor, USA) and fermentation extracts were obtained by pressing the PUF under vacuum. The pressed PUF was washed with 1.5 l distilled water to remove any remaining substrate and the wash water was filtered through a 0.2 μm pre-weighed filter (Gelman, Ann Arbor, USA). Both the PUF and filters were dried for 4 days at 65°C for biomass determination.

3.6. Sample treatment for sporulation studies

Separate samples were used to study the sporulation patterns, 250 ml 0.2% Tween 80 was added to the PUF and the mixture was stomachered for exactly 5 min. Extraction of the liquid in the PUF took place under vacuum in a Buchner funnel. The same was repeated once more and the 500 ml Tween solution was collected to count the number of spores using an improved Neubauer counting chamber.

3.7. Tannase assay

An amount of 50 μl of the fermentation extracts were added to 1 ml of a 0.3 mM solution of tannic acid in 0.1 M acetate buffer (pH 5). After incubation at 30°C for 30 min, the reactions were stopped by addition of 0.2 ml HCl (2 M). Blanks were produced by adding 50 μl of enzyme extract to the same reaction mixture already containing the HCl. The quantity of gallic acid released during hydrolysis of tannic acid represents the tannase activity, which was expressed in micromole of gallic acid per millilitre of fermentation extract per minute. Gallic acid was measured with the rhodanine reaction. Rhodanine (2-thioxo-4-thiazolidinone) was purchased from ICN (Thame, UK). To 100 μl of reaction mixtures and blanks 150 μl of rhodanine solution (0.667% (w/v) in methanol) was added and the mixtures were vortexed. After exactly 5 min, 2.25 ml of aqueous KOH (0.5 M) solution was added and 20 min afterwards, the absorbance was read at 520 nm. Reactions were carried out at room temperature. A standard curve was obtained by determining in triplicate the gallic acid content of standard solutions with concentrations ranging from 0 to 0.20 g/l gallic acid.

3.8. Determination of tannic acid with rhodanine

The concentration of tannic acid in the fermentation extracts was determined with the method of Inoue and Hagerman [16]. To 2.5 ml of 1 M H_2SO_4 was added to 0.5 ml fermentation extract and the reaction mixture was incubated at 100°C for 26 h. After acid hydrolysis, the hydrolysate was diluted with the appropriate amount of water and the concentration of gallic acid was determined with the rhodanine reaction as described before. Controls were produced by measuring the gallic acid concentrations of the original fermentation extracts without being subjected to hydrolysis. The molar concentration of tannic acid is represented by the amount of esterified gallic acid and therefore equals the gallic acid in the reaction mixtures minus the gallic acid in the controls.

3.9. Residence time distribution (RTD) studies and solids mixing modelling

With the reactor in its original configuration at an inclination angle of 4° and a rotational speed of 0.7 rpm, a step change experiment was carried out as described by Levenspiel [17]. Steady flows of red PUF cubes through the cylinder were established at flow rates of 300 and 600 ml per day and at a given time the feed was changed instantaneously from red to blue PUF cubes, those being the tracer. The percentage of blue cubes leaving the vessel was measured against elapsed time. The mean residence times were determined by measuring the area under the concentration–time curve. Modelling of the solids mixing and the prediction of washout conditions in the fermenter has been done using an adaptive Runge–Kutta algorithm with MathCAD, version 2000.

4. Results and discussion

4.1. Physiological studies in static and rotating batch culture

The effect of rotation on fungal growth, sporulation and tannase productivity was studied by comparing those parameters in static and rotating batch fermentations. The biomass yield in static culture was 0.39 g biomass/g tannic acid as opposed to 0.24 g biomass/g tannic acid in rotating culture. Mycelial damage is thought to have caused the lower biomass yield. Fig. 2 shows that the fungus in rotating culture stops growing after 24 h. Also the rate of sporulation and the final number of spores formed are lower than in static culture (Fig. 3).

In static culture, the fungus started sporulating at around 36 h of fermentation. Environmental factors such as light, presence of oxygen, relative humidity and temperature can be important for the initiation of sporulation. Although the formation of spores is more often induced by conditions of nutrient limitation, commonly nitrogen limitation, sometimes, depending on the fungus and on the precise conditions, it may be induced by limitation in the supply of the carbon source or another nutrient [18]. In these experiments

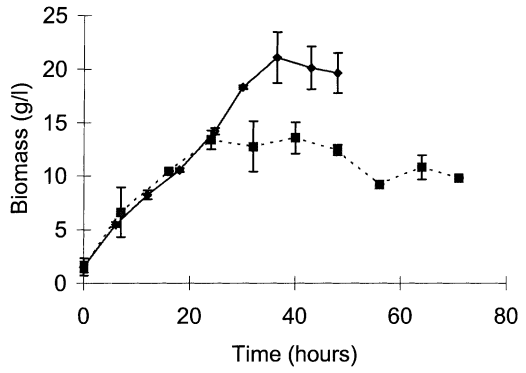

Fig. 2. Biomass formation in static (—◆—) and rotating (- - -■- - -) batch SSF with *P. glabrum*. Biomass was expressed as g of biomass/l of fermentation extract. Values are the mean ± S.D. of duplicate cultures.

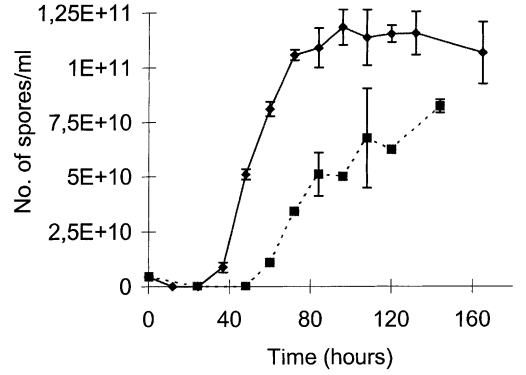

Fig. 3. Spore formation in static (—◆—) and rotating (- - -■- - -) batch SSF with *P. glabrum*. Spore counts were expressed as the number of spores/ml of fermentation extract. Values are the mean ± S.D. of duplicate cultures.

it is believed that tannic acid limitation was the inducer for sporulation: measurements of the tannic acid concentration in static batch culture showed that the fungus ran out of its main carbon source after approximately 32 h (Fig. 4). It should be noted that the fungus first hydrolyses the tannic acid to gallic acid, catalysed by the extracellular tannase. The gallic acid is then metabolised by the fungus. In the rotating SSF, this occurred after 32 h and the first increase in number of spores was observed after 42 h (Fig. 3). The kinetic aspects of this are currently being studied. We believe that the kinetics of substrate uptake and fungal growth can be modelled as a two-stage process involving the enzyme catalysed hydrolysis of tannic acid followed by fungal growth on the gallic acid resulting from the first stage.

The effect of rotation on SSF productivity and sporulation has been studied extensively but not much is known about the effects of rotation on the type of fungal damage. High rotational speeds increase the frequency of particle tumbling and this movement can disrupt the mycelium in the early growth stages [8]. Silman [12] suggested that mechanical scraping of mycelia interferes with spore formation by breaking either the conidiophore, the swollen vesicle, or the phialides of the spore-forming structure of the fungus, i.e. aerial hyphae are crushed onto the substrate surface during agitation thereby inhibiting sporulation. Stuart et al. [9] found that the biomass produced in a rotating drum bioreactor was easily peeled off the substrate surface and therefore suggested that shear forces broke penetrative hyphae from surface mycelia or pulled them out of the substrate itself. The effect of shear forces on morphology caused by agitation in submerged fermentation has received much more attention. A high degree of agitation can change the form in which the fungus is growing both by direct breakage of the hyphae largely on the septa or by changing the development of morphology. Mechanical forces can lead to vacuolation of older hyphal compartments, which may lead to autolysis or weakened hyphae, accelerating hyphal fragmentation [19]. These processes are dependent on hyphal age, physiological state and fungal species [19,20]. Again, further study of the aspects is needed since in our case the fungus is largely shielded from the direct effects of shear and agitation by its PUF environment.

The low biomass yield in rotating culture also had an effect on the tannase productivity. As can be seen in Fig. 5, higher titres of extracellular tannase were obtained in static batch than in rotating batch culture. High activities of the

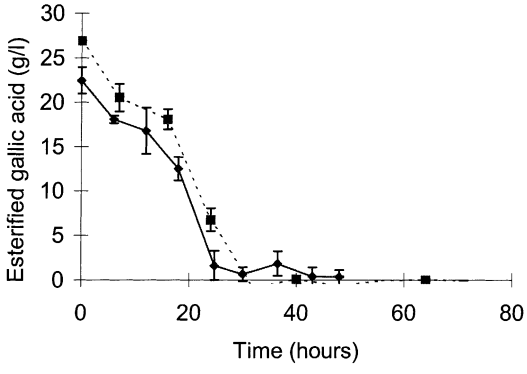

Fig. 4. Esterified gallic acid in static (—◆—) and rotating (- - -■- - -) batch SSF with *P. glabrum*. Tannic acid is expressed as the amount of gallic acid released from tannic acid/l of fermentation extract. Values are the mean ± S.D. of duplicate determinations.

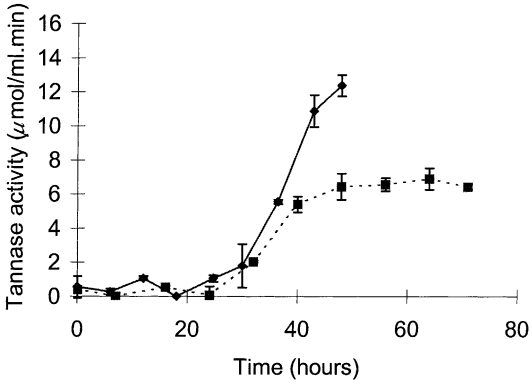

Fig. 5. Extracellular tannase activities in static (—◆—) and rotating (- - -■- - -) batch SSF with *P. glabrum*. Values are the mean ± S.D. of duplicate determinations.

enzyme could be measured only after 24 h; the evidence is that tannase formation was not directly growth related. However, the main substrate tannic acid was utilised within 24 h of fermentation whilst very little tannase activity could be seen. As noted above the microorganism produces a basic level of tannase that hydrolyses tannic acid to glucose and gallic acid; it appears that the gallic acid functions as an inducer. It is also noteworthy that extracellular tannase cannot be detected in submerged (i.e. liquid) fermentation of the same organism on tannic acid.

4.2. Solids mixing

During the flow through the bioreactor the PUF cubes take different routes and therefore spend different lengths of time in the vessel. The distribution of the time for the PUF cubes to leave the vessel is the residence time distribution (RTD). Fig. 6a and b represent the RTD in the fermenter at flow rates of 300 and 600 ml PUF cubes per day. The calculated average residence time of the material were 2.54 and 4.57 days for flow rates of 600 and 300 ml per day, respectively.

The experimental data are compared in the figure below to the RTD of a vessel with perfect mixing. At these flow rates the early stages of the RTD show some evidence of plug flow (corresponding to a time delay of around 0.5 day). At longer residence times there is again some divergence between the tails of the experimental and perfectly mixed profiles. The shapes of the experimental profiles suggest that the RTD might be fitted by a dispersed plug flow model.

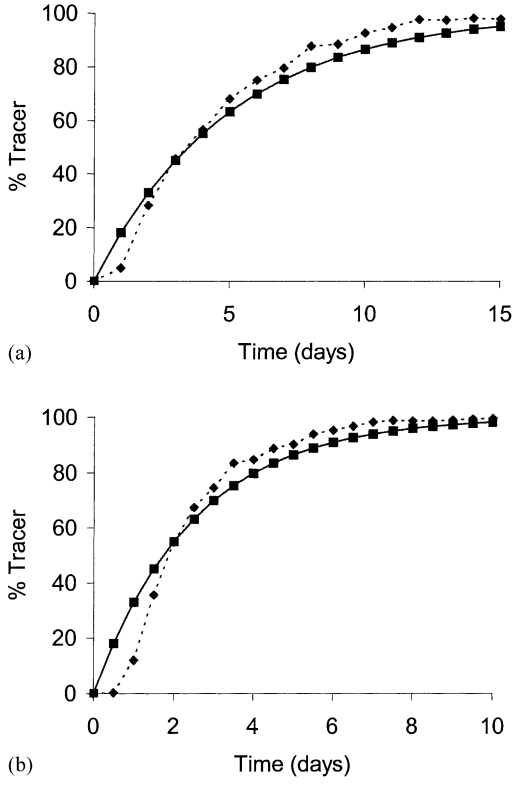

Fig. 6. Experimental (---◆---): % blue PUF and theoretical data (—■—): perfect mixing representing the flow pattern in the fermenter at flow rates of (a) 300 ml and (b) 600 ml PUF cubes per day.

The extent of solids mixing is particularly important in relation to the development of a continuous SSF process without re-inoculation, i.e. with a "sterile" feed. A necessary condition that must be satisfied if the system is to work at all is that there must be sufficient back mixing in the system to ensure that the biomass concentration at any point in the fermenter does not fall with time. If the solids are in pure plug flow, for example, the only way this condition can be satisfied is by continuous re-inoculation of the feed. For example, consider the fate of a short slug of inoculated feed in the middle of otherwise sterile feed: biomass will grow as long as substrate is available but in the absence of axial mixing it will remain confined to the initial slug volume. Thus, the biomass concentration in this slug will change in time (and space) as if it were a batch process.

There are probably two mechanisms of fungal back mixing in SSF system. The first is by spore transport (which can be air-borne). The second is by transfer of the fungi with the solid matrix. In this work we concentrate on the latter mechanism since we can quantify the mixing of the PUF particles, although we recognise that spore transport cannot be disregarded (and a similar model to that described below may also be appropriate).

One way to characterise solids mixing in an SSF fermenter is to use one of the classical chemical engineering approaches that have been employed in conventional reactor engineering. For example, the system could be characterised through its experimentally determined RTD, and/or by a string of stirred tanks model, or by a dispersed axial flow model. In the latter case the key mixing parameter is the Peclet number. Alternatively, a more mechanistic approach could be employed. Two different approaches will be briefly discussed here.

4.3. Dispersion model

In this approach we assume that the solids mixing can be described by an axial dispersion coefficient, which must be determined experimentally. If we assume that the fungal biomass is attached to the solid support (containing the substrate), it is then consistent to use the same dispersion coefficient D_L to describe the deviation from plug flow for both biomass and substrate.

Assuming Monod growth kinetics and a constant yield coefficient (Y_{xs}) we can write steady-state balances on biomass (x) and limiting substrate. We assume constant mean axial velocity u. With sterile feed the biomass concentration at any point, z in the reactor is related to the substrate concentration at that point, s and in the feed (s_0) by $x = Y_{xs}(s_0 - s)$; a substrate balance leads to

$$D_L \frac{d^2 s}{dz^2} - u\frac{ds}{dz} - \frac{\mu_m s(s_0 - s)}{K_s + s} = 0 \quad (1)$$

where μ_m is the maximum specific growth rate and K_s the saturation constant.

With (Danckwerts) boundary conditions: at the inlet ($z = 0$)

$$s_0 = s - \frac{D_L}{u}\frac{ds}{dz} = 0 \quad (2)$$

and exit ($z = L$)

$$\frac{ds}{dz} = 0$$

These equations can be put into non-dimensional form, viz.

$$\frac{1}{Pe}\frac{d^2y}{dw^2} - \frac{dy}{dw} - \frac{NO(1-y)y}{K+y} = 0 \quad (3)$$

with boundary conditions: at $w = 0$

$$1 = y - \frac{1}{Pe}\frac{dy}{dw}$$

and $w = 1$

$$\frac{dy}{dw} = 0$$

where $y = s/s_0$, $w = z/L$, $NO = L\mu_m/u$, $K = K_s/s_0$ and $Pe = uL/D_L$. L is the length of the vessel. The dimensionless group NO is the product of the mean residence time $\tau = L/u$ or V/F where V is the active reactor volume and F the volumetric feedrate) and the maximum specific growth rate; alternatively it can be seen to be the ratio of the maximum specific growth rate to the nominal dilution rate D. Pe is the Peclet number.

4.4. Washout condition

As noted above, in order for a fermenter with sterile feed to operate steadily and continuously, it is always necessary for the mean residence time of material in the fermenter to be greater than some critical (washout) value which depends on the growth kinetics and the extent of mixing in the system. In pure plug flow, i.e. with no back mixing there is no finite residence time to satisfy this condition. In the case of a perfectly mixed fermenter the dilution rate at washout, D_w, i.e. the condition where no substrate is converted into biomass is given by

$$D_w = \frac{\mu_m s_0}{K_s + s_0} \quad (4)$$

or, in dimensionless form

$$\frac{\mu_m}{D_w} = \tau_w \mu_m = NO_w = 1 + K \quad (5)$$

where τ is the residence time and the subscript "w" refers to the limiting washout condition. These relations should correspond to the limit as $Pe \to 0$.

At the other limit where there is no mixing, i.e. $Pe \to \infty$, the critical (washout) residence time and dilution rate are ∞ and 0, respectively.

The washout conditions for a system described with the dispersion model have been analysed by Fan et al. [21] for small dispersion coefficients (i.e. large Pe). Neglecting the maintenance term in the biomass growth kinetics, their expression for washout is

$$NO_w = \tau_w \mu_m = \frac{\mu_m}{D_w} = 0.25\,Pe(1+K) \quad (6)$$

Whilst it does not seem possible to find an analytical solution for the critical value of NO for the whole range of Peclet numbers, it is possible to compute the washout conditions by solving the dispersion equation (Eq. (3)) and its associated boundary conditions for given Pe and K and iterating to find the critical value of NO for which there is no change in substrate concentration (i.e. no biomass growth). Some results are shown in Fig. 7a and b: computed curves are shown for $K = 0.1$ and 0.001, respectively. The computations are also compared with Fan et al. [21] approximate solution (dotted lines). The feasible operating region — that is where there is no washout — is above the curves. It will be seen that Fan et al. [21] predictions begin to diverge significantly from the computed curves for Peclet numbers below around 10; they are not valid for lower Peclet numbers. The numerical calculations tend to the perfectly mixed solution (Eqs. (4) and (5)) for low Peclet numbers. For high Peclet numbers the numerical solutions become

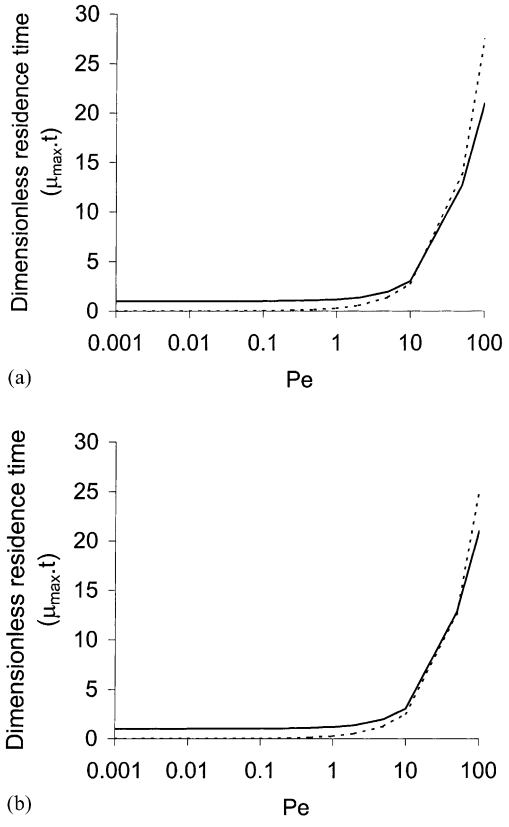

Fig. 7. Computed (——) and analytical [21] (· · ·) washout conditions for (a) $K = 0.1$ and (b) 0.001, respectively.

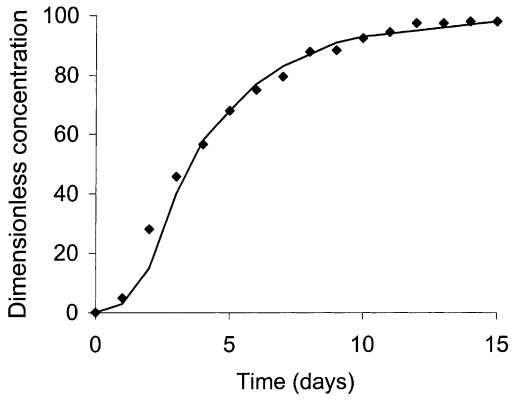

Fig. 8. Comparison of the experimental residence time distribution (◆) at a flow rate of 300 ml per day and the predicted residence time distribution (——) obtained from the dispersion model with $Pe = 2$.

very sensitive to *Pe*. As expected, higher values of *K* lead to slightly lower critical dilution rates (i.e. higher NO) over the whole range of mixing conditions, but the answers are not particularly sensitive to *K*, particularly if the accuracy of typical kinetic data and models is taken into account.

The RTD data described above can be interpreted in terms of a dispersion model. In Fig. 8 the experimental RTD in the SSF (expressed as an "F curve" [17]) is compared with the dispersion model: a Peclet number of around 2 gives a reasonable fit to the data, although there is divergence between the model and the experimental data in the tail region. A Peclet number of this order of magnitude suggests that the flow is much closer to perfect mixing than plug flow; it is also, unfortunately, a region where the use of dispersion models becomes questionable [17] and where the choice of boundary conditions is critical. Leaving these qualification aside, the predicted washout conditions for $Pe = 2$ are NO = 1.35 and 1.49 for $K = 0.001$ and 0.1, respectively. In other words, the minimum residence time for sustainable continuous fermentation is not 1/(specific growth rate) but between 1.35 and 1.5 times this, because of the deviation from perfect mixing.

4.5. An approach to mechanistic modelling

In the previous section we followed other workers in attempting to describe the solids mixing performance in a solids transport reactor or contactor by axial dispersion models. As noted, it is questionable whether a dispersion model is really appropriate under the conditions of our experiments where the deviations from plug flow are large. Moreover, it is hard to justify or interpret such models in terms of the physics of the stick-slip, tumbling and avalanching behaviour characteristic of solids motion in rotating devices [22]. These arguments suggest that an alternative, preferably mechanistic, approach should be developed. We have developed a model, to be reported elsewhere, in which the reactor is modelled as a moving series of cells or compartments with forwards and backwards exchange between the cells. Physically the compartments represent the zones established by the internal baffles (or screw pitch in the case of a reactor with an internal archimedian screw). Although the solids exchange between compartments cannot be predicted from first principles, the exchange parameters can be estimated from RTD data.

5. Conclusions and future work

Lower biomass and tannase yields were obtained in rotating batch culture at 0.7 rpm compared to static culture presumably due to the deleterious effect of shear. Also the rate of sporulation and the final number of spores formed were lower than in static culture. Studies have to be conducted to elucidate whether lower rotational speeds can increase the yields and the rate of sporulation while at the same time remaining the well-mixed conditions in the vessel.

Operating the solid-state fermenter at flow rates of 300 and 600 ml PUF cubes per day and a rotational speed of 0.7 rpm provided a mixing pattern with near perfect mixed flow. The solids mixing in the system can be characterised using a dispersion model which can then be used to predict the washout condition in the reactor. It was suggested that the minimum residence time for a successful continuous fermentation is approximately 1.5/(maximum specific growth rate) of the fungus. Taking this into account, experiments on a continuous fermenter are now being carried out: we have achieved continuous growth over a period of around six residence times and will report the detailed results at a later date.

Besides sufficient back mixing, spore transfer is also essential for inoculation of the sterile feed entering the reactor. Prediction of operating variables of the solid-state fermenter is difficult since little is known about the requirements for effective spore transfer. More research is needed to elucidate the amount of particle contact and number of transferable spores needed for the successful operation of the continuous process.

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