Biomass production of *Aspergillus fumigatus* on spent sulphite liquor under non-aseptic conditions

WA Pretorius* and GG Lempert
Department of Chemical and Environmental Engineering, University of Pretoria, Pretoria 0002, South Africa

Abstract

Aspergillus fumigatus was grown on spent sulphite liquor (SSL) in three different reactor configurations to determine which configuration required the minimum feed dilution. Stable monoculture growth under non-aseptic conditions could only be maintained in a continuously stirred tank reactor (CSTR) with a microscreen as cell separator and in a selector/producer reactor chain. The reactor chain required the least feed dilution. Monod kinetics could be used to describe the reactor performance with *A. fumigatus*.

Introduction

Aspergillus fumigatus was selected and cultivated on highly diluted spent sulphite liquor (SSL) using the microscreen process (Pretorius and Lempert, 1993 a; b). This thermotolerant fungus, if continuously grown as a monoculture for single-cell protein (SCP) production, has distinct advantages above fungi which only grow in the mesophilic temperature range. With an optimum growth at 45°C continuous cultivation of this fungus for SCP production could be more economical than with mesophilic fungi, because less biologically generated heat needs to be removed and cooling of the relatively high discharge temperature of the SSL effluent is unnecessary (Pretorius and Lempert, 1993 b).

These properties of the effluent and fungus contribute positively towards the economic viability of the commercial production of *A. fumigatus* on SSL. Due to increased viscosity and decrease in oxygen transfer rates in broths containing more than about 10 $g \cdot \ell^{-1}$ (dried cells) of filamentous micro-organisms (Wille, 1992) the microscreen process as applied here is essentially limited to substrate concentrations of less than 4 000 $mg \cdot \ell^{-1}$ biodegradable chemical oxygen demand (COD). This means that the already highly diluted effluent (biodegradable COD ≈ 6 to 9 g $COD \cdot \ell^{-1}$) should be further diluted with clean water, an action that has a detrimental effect on the economics of the process.

Various reactor and process configurations are possible which will allow the cultivation of *A. fumigatus* on SSL effluent without any additional dilution. In this paper two reactor configurations are compared as to their suitability for the cultivation of *A. fumigatus* on SSL effluent with a reduced need for additional dilution.

Theoretical considerations

In continuously fed suspended growth bioreactors, different flow configurations are available for different applications. The two configurations most often employed in biological wastewater treatment can be compared with a continuously stirred tank reactor (CSTR) without and with cell recycle (Grady and Lim, 1980).

In a CSTR without cell recycle, the cell age (Θ_c) and hydraulic residence time (τ) are the same. As the mass of cells produced per mass of COD utilised (i.e. the yield, Y) is generally less than unity, the cell concentration (X) in the reactor is always less than the inflow biodegradable organic substrate concentration (S_0). Unfortunately the selective pressure (Pretorius, 1987) of the microscreen process is lost in such a flow configuration.

In a CSTR with cell recycle, Θ_c and τ are separately controlled and the reactor is always operated with $\Theta_c > \tau$. The result is that X is also greater than S_0. The microscreen process can be considered as a CSTR with cell recycle. When *A. fumigatus* as selected micro-organism (with Y = 0,7; Pretorius and Lempert, 1993 b) is grown with SSL as substrate, having a biodegradable COD (S_0) of between 6 and 9 $g \cdot \ell^{-1}$, X usually exceeds 10 $g \cdot \ell^{-1}$. At this biomass concentration, however, the oxygen transfer efficiency is seriously limited (Wille, 1992).

By combining the microscreen process in a two-stage flow configuration with a CSTR without cell recycle, the benefit of the selection pressure of the microscreen process is retained while the diminishing need for dilution of S_0 for a CSTR without cell recycle is affected. The mathematical modelling of CSTRs in series configurations is fully covered by Grady and Lim (1980).

Materials and methods

Bioreactor types

Two identical reactors were used, one with an unrestricted constant volume outflow (CSTR without cell recycle) and one equipped with a 100 µm pore size crossflow-microscreen (Kühn and Pretorius, 1989) on the constant volume outlet (CSTR with cell recycle). Each of these reactors was provided with their own variable rate feed supply pumps, air supply and temperature controllers. Biomass harvesting on the microscreen bioreactor was done with a variable speed pump. The two reactors are shown schematically in Fig. 1.

Flow arrangements

The performance of the reactors when operated in three different configurations was evaluated, namely a CSTR without cell recycle (Fig. 1a), a CSTR with cell recycle (Fig. 1b) and a chain of a CSTR with cell recycle followed by a CSTR without cell recycle (combination of Figs. 1a and 1b) as shown schematically in Fig. 2.

*To whom all correspondence should be addressed
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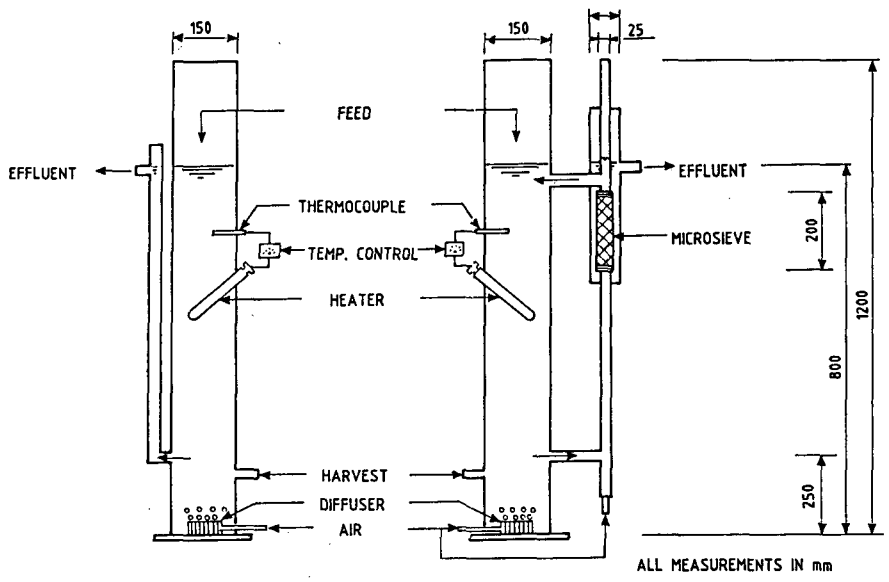

Figure 1
(a) CSTR without cell recycle and (b) CSTR with cell recycle

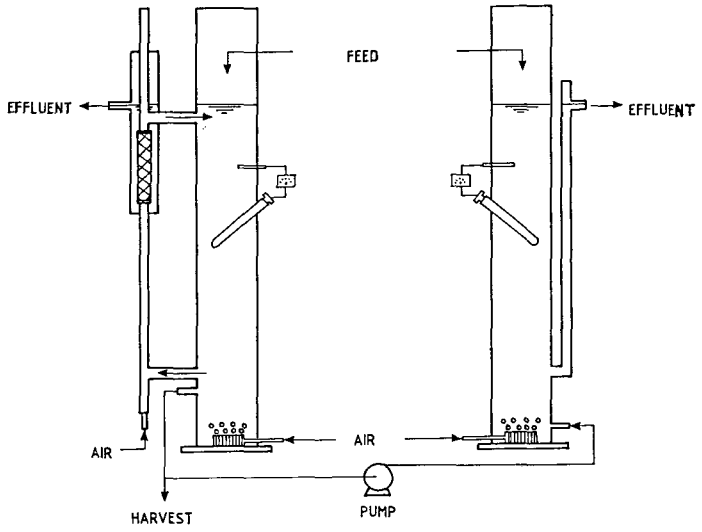

Figure 2
CSTRs in series: (a) CSTR with cell recycle and (b) CSTR without cell recycle

Feed

Spent sulphite liquor, supplemented with nutrients (Tabak and Cooke, 1968) was used as feed. No additional treatment (e.g. steam stripping) was applied to the SSL prior to feeding. Undiluted SSL (i.e. 6 to 9 $g \cdot \ell^{-1}$ biodegradable COD) was used for the CSTRs without cell recycle, while diluted SSL (2,5 to 3,5 $g \cdot \ell^{-1}$ biodegradable COD) was used for the CSTRs with cell recycle.

Inoculum

All reactors were initially inoculated with screen-dewatered monocultures of *A. fumigatus* obtained from a selector (micro-screened) reactor (Pretorius and Lempert, 1993b). The initial biomass concentration was approximately 4 $g \cdot \ell^{-1}$ dry mass.

Operation and sampling

The temperature and pH were kept constant at 45°C and 5,8 respectively while the hydraulic residence times (τ) and/or cell ages (Θ_c) were varied for the various bioreactors as shown in Table 1. Two operation modes were followed with the chain

TABLE 1
τ AND Θ_c VARIATIONS FOR THE DIFFERENT BIOREACTORS

Bioreactor	τ and Θ_c range		Appr. step incr. (h)
	min (h)	max (h)	
CSTR without cell recycle	$\tau = \Theta_c = 2$	$\tau = \Theta_c = 8,5$	1
CSTR with cell recycle	$\tau = 3$, $\Theta_c = 4$	$\tau = 3$, $\Theta_c = 19$	1 to 2
CSTRs in series:			
First reactor	$\tau = 2,8$, $\Theta_c = 12$	$\tau = 2,8$, $\Theta_c = 12$	None
Second reactor	$\tau = \Theta_c = 2$	$\tau = \Theta_c = 10$	1

TABLE 2
PERFORMANCE OF A SINGLE CSTR WITH CELL RECYCLE WITH VARYING CELL AGES (Θ_c)

τ	Θ_c	S_o	S	X	X*
(h)	(h)	(mg·ℓ$^{-1}$ COD)		(g·ℓ$^{-1}$ biomass)	
2,63	4,13	2 515	449	2,73	2,21
2,65	4,46	2 829	568	2,93	2,58
2,71	5,08	2 633	606	2,15	2,55
2,75	5,63	2 436	214	2,60	3,03
2,88	6,24	2 705	894	2,94	2,56
2,84	8,56	2 810	782	4,07	3,91
2,76	9,15	2 989	509	5,39	5,21

* X calculated from kinetic constants (Pretorius and Lempert, 1993 b) using equations from Grady and Lim (1980).

reactors. In the first operation mode all the harvested biomass of the first reactor was supplied to the second reactor, while in the second mode only half of the harvested biomass was supplied to the second reactor.

After changing any operating parameter, the operating conditions were kept constant for at least three cell ages to stabilise and reach steady state conditions before the first duplicate sample was taken. Steady state conditions were assumed when the COD and suspended solids concentration between two samples, with one cell age lapse in between the sampling period, was within 5% of each other. Another two samples (also in duplicate) were then taken with at least one cell age lapse between samples, i.e. one data point comprised six samples at steady state conditions.

COD analyses on the feed and different filtered effluents and suspended solids were determined according to *Standard Methods* (1985).

Results and discussion

Single CSTR without cell recycle

Although the inoculum was essentially bacteria-(and yeast-) free (Pretorius and Lempert, 1993b), excessive contamination with a corresponding loss of *A. fumigatus* was observed at all hydraulic residence times tested. The selection pressure exerted by the elevated temperature (45°C) alone was not enough to maintain a monoculture of *A. fumigatus* and the fungus was essentially replaced by bacteria after prolonged operation at any of the attempted hydraulic residence times. Although the cell concentration initially (before excessive bacterial contamination occurred) closely followed the predicted values, this mode of operation was not suitable for the non-aseptic cultivation of *A. fumigatus*.

Single CSTR with cell recycle

The single CSTR with cell recycle maintained a monoculture with no bacterial (or yeast) contamination at the different cell ages tested. Typical steady state data are shown in Table 2.

The data in Table 2 show that the kinetic constants and the Monod kinetic model can be used to predict the biomass (and other) concentrations. Although additional dilution of the SSL effluent was necessary, the CSTR with microscreen as cell separator produced a stable selective system where *A. fumigatus* could indefinitely be maintained as a monoculture under non-aseptic conditions.

Reactors in series operation

By using the CSTR with cell recycle as a selector reactor (with diluted feed) and the CSTR without cell recycle (with undiluted feed) as a biomass production reactor, a stable process for the production of a monoculture of *A. fumigatus* was maintained with both the full and half harvest flow rate from the first reactor, provided τ (and Θ_c) in the producer reactor did not exceed approximately 8 h. At this operating range, the selector reactor contained no bacteria or yeasts, whereas contamination in the producer reactor due to bacteria or yeasts never exceeded 10% of the total biomass. Typical process data are shown in Table 3.

The data in Table 3 show a good correlation between actual and calculated biomass concentrations. Furthermore, very good substrate removals occurred in the production reactor at hydraulic residence times of greater than 4 h. This was observed for both the full and half harvest stream inoculated into the producer reactor. This means that the savings in dilution water will depend on the maximum volume ratio of the selector to the producer reactor that will allow acceptable levels of bacterial contamination in the producer reactor.

Conclusions

- No dilution water savings could be achieved in a CSTR without cell recycle due to excessive bacterial contamination and the eventual loss of the *A. fumigatus* culture upon prolonged operation.
- A CSTR with microscreen as cell separator could maintain indefinitely a monoculture of *A. fumigatus* on SSL effluent. The SSL effluent (feed) must, however, be diluted to limit the biomass concentration to levels where oxygen transfer is not impaired.
- A combination of a selector and producer reactor in a series produced a stable process for maintaining a monoculture of *A. fumigatus* with a reduced need for dilution water.
- The Monod kinetic model and the evaluated kinetic constants could be used to predict the performance of *A. fumigatus* in a variety of reactor configurations.

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References

GRADY, CPL and LIM, HC (1980) *Biological Wastewater Treatment*. Marcel Dekker, Inc., New York.

KÜHN, AL and PRETORIUS, WA (1989) Fungal purification of an industrial effluent containing volatile fatty acids by means of a crossflow-microscreen technique. *Water Sci. Technol.* **21** 221-229.

PRETORIUS, WA (1987) A conceptual basis for microbial selection in biological wastewater treatment. *Water Res.* **21**(8) 891-894.

PRETORIUS, WA and LEMPERT, GG (1993a) The selective cultivation of the thermotolerant *Aspergillus* sp. on spent sulphite liquor. *Water SA* **19**(2) 69-72.

PRETORIUS, WA and LEMPERT, GG (1993 b) Growth characteristics

TABLE 3
PERFORMANCE OF A SELECTOR-PRODUCER REACTOR CHAIN

Reactor	τ (h)	Θ_c (h)	S_o (mg·ℓ$^{-1}$ COD)	S (mg·ℓ$^{-1}$ COD)	X (g·ℓ$^{-1}$ biomass)	X* (g·ℓ$^{-1}$ biomass)
Selector reactor	2,78	9,15	2 980	510	5,42	5,22
Producer reactor						
(a) Full harvest flow	1,95	1,95	6 340	5 705	0,46	0,46
	2,87	2,87	6 340	4 240	1,40	1,45
	3,05	3,05	6 320	4 090	1,43	1,46
	4,12	4,12	6 040	528	4,28	4,10
	6,14	6,14	5 965	206	4,37	4,33
(b) Half harvest flow	7,09	7,09	7 109	237	4,38	4,57
	7,52	7,52	6 400	165	4,34	4,40
	8,74	8,74	6 140	135	4,30	4,22
	9,81	9,81	7 032	122	4,78	4,66

* X calculated from kinetic constants (Pretorius and Lempert, 1992 b) using equations from Grady and Lim (1980).

of *Aspergillus* sp. grown on spent sulphite liquor. *Water SA* **19**(2) 73-76.

STANDARD METHODS (1985) *Standard Methods for the Examining of Water and Wastewater*; Ed. MAH Franson (16th edn.) American Public Health Ass. Washington, DC.

TABAK, HH and COOKE, WB (1968) Growth and metabolism of fungi in an atmosphere of nitrogen. *Mycologia* **60** 115-140.

WILLE, P (1992) Optimering van Suurstofoordrag en Sifontwerp in 'n Nie-Newtoniese Suspensie van Filamentagtige Fungi. Ph.D. Thesis, Department of Chemical and Environmental Engineering, University of Pretoria. (In preparation).