Growth characteristics of *Aspergillus* sp. grown on spent sulphite liquor

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Abstract

The growth characteristics of *Aspergillus* sp. when grown on spent sulphite liquors (SSL), were determined. Maximum sustainable growth occurs at temperatures < 50°C with near optimum growth at temperatures < 45°C. No growth occurs at pH < 4,5 with an optimum pH between 5,5 and 6. The biodegradable fraction of SSL with a monoculture of *Aspergillus* sp. was 33,6%. The growth kinetic constants of *Aspergillus sp.* when grown on diluted SSL at 45°C were:

μ_{max} = 0,318 h^{-1}; K_s = 260 mg COD·ℓ^{-1};
b = 0,016 h^{-1} and Y = 0,7 g biomass·(g COD)$^{-1}$.

The crude protein contents and amino acid profile of *Aspergillus* sp. are given.

Introduction

In selecting micro-organisms suitable for single-cell protein (SCP) production from spent sulphite liquor (SSL) at 45°C, *Aspergillus* sp., most probably *A. fumigatus,* was the dominating species (Pretorius and Lempert, 1993). *A. fumigatus,* a very commonly occurring fungus, is easily identifiable and inhabits most places on earth. It is also an opportunistic pathogen and the most important causal agent of systemic mycosis. The infectious phase is mainly conidiospores and the sites of attack are usually the lungs and the respiratory tract (Domsch et al., 1980).

If the intention is to mass-cultivate *A. fumigatus* for SCP purposes, it would be important to know its growth characteristics and whether or not conidiospore formation generally occurs during continuous cultivation.

In this paper some factors that could affect the mass cultivation of *Aspergillus* sp. as well as its amino acid composition as a potential SCP source are examined.

Materials and methods

Reactor configuration and substrate

The experimental reactor set-up and diluted SSL substrate used was as described elsewhere (Pretorius and Lempert, 1993).

Evaluation of temperature and pH effects on the growth rate of *Aspergillus* sp.

To evaluate the effects of temperature and pH on the growth rate of *Aspergillus* sp., the general operating conditions were fixed: Substrate COD concentration at 10 g·ℓ^{-1}; hydraulic residence time (τ) at 3 h and cell residence time (Θ_c) at 9 h.

As a reference point the reactor was operated at a temperature of 45°C and a pH of 5,5. Once steady state growth was obtained the test parameter was stepwise increased (or decreased) as shown in Table 1.

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The temperature and pH were maintained at any particular set point with the thermostat and pH-stat respectively. To lower the pH, 5N H_2SO_4 was used and to increase the pH, 5N NaOH was used.

After each step change the reactor was operated for three cell residence times to ensure steady state conditions.

Determination of the biodegradable fraction of SSL

The method of Grady and Lim (1980) was used to determine the inert and biodegradable fractions of SSL. A 15 ℓ temperature-controlled (45°C) and aerated batch reactor filled with diluted SSL substrate at pH 5,5 was inoculated with a pure culture of *Aspergillus* sp. Two hundred and fifty mℓ samples were taken at 2 h intervals for the 48 h duration of the experiment. Compensation for evaporation was made with distilled water. The samples were filtered and analysed for COD.

Determination of kinetic constants of *Aspergillus* sp. grown on SSL

To determine the kinetic constants, τ was fixed between 2,5 and 3,3 h, pH at 5,5 and temperature at 45°C. Θ_c was varied from 18 h (where dissolved oxygen was limiting) till the biomass was wasted faster than growth (washout). Three cell residence times were allowed between consecutive step changes. The results were analysed by the methods proposed by Grady and Lim (1980).

Analytical methods

Flow rates: The volumes of feed used and effluent and biomass produced were collected for each cell residence period and from these the respective flow and biomass harvesting rates were calculated. COD analyses were done on the feed and filtered effluents and the suspended solids were determined according to *Standard Methods* (1985).

Protein content: Washed, freeze-dried biomass (cultivated at Θ_c = 9 h, τ = 3 h, T = 45°C and pH = 5,5) was used for protein analysis. Crude protein was determined in duplicate by the micro-Kjeldahl method (Horwitz, 1975) and the amino acid profile on acid digested samples analysed on a Beckman 121 M

TABLE 1
STEPWISE CHANGES IN PARAMETERS

Parameter varied	Range		Step	Fixed parameter and value
	Minimum	Maximum		
Temperature (°C)	40	50	2,5	pH = 5,5
pH	4,0	7,5	0,5	Temp = 45°C

amino acid analyser. Microscopic observations were done on live cultures under phase contrast illumination.

Results

Effect of temperature

The effect of temperature on the biomass production (growth rate) of *Aspergillus* sp. is shown in Fig. 1.

Whereas essentially a monoculture of possibly *A. fumigatus* was microscopically observed and verified on streaked-plate agar cultures at temperatures of 45°C and above, more than one filamentous fungus were observed at temperatures < 43°C. No conidiospore formation was ever observed at any temperature in the continuous culture reactors. At 40°C conidiogenous structures which differed significantly from normally observed *A. fumigatus* were observed (Fig. 2).

Effect of pH

The effect of pH on the biomass production rate is shown in Fig. 3.

At pH values between 5 and 5,8 microscopically observed mycelia appeared 'healthy' with little or no vacuoli. Outside this pH range the mycelia appeared thicker, granier and with an abundance of vacuoli. At pH values above 6,5 bacterial contamination became excessive.

Non-biodegradable fraction of SSL

When plotting the substrate removal rate (q) against the soluble COD concentration (Grady and Lim, 1980) it was found that *Aspergillus* sp. could degrade only 33,6% of the SSL-COD. A

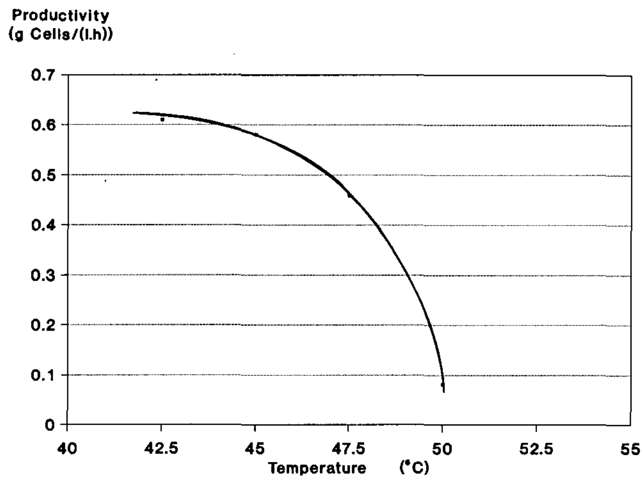

Figure 1
Effect of temperature on the production rate of A. fumigatus

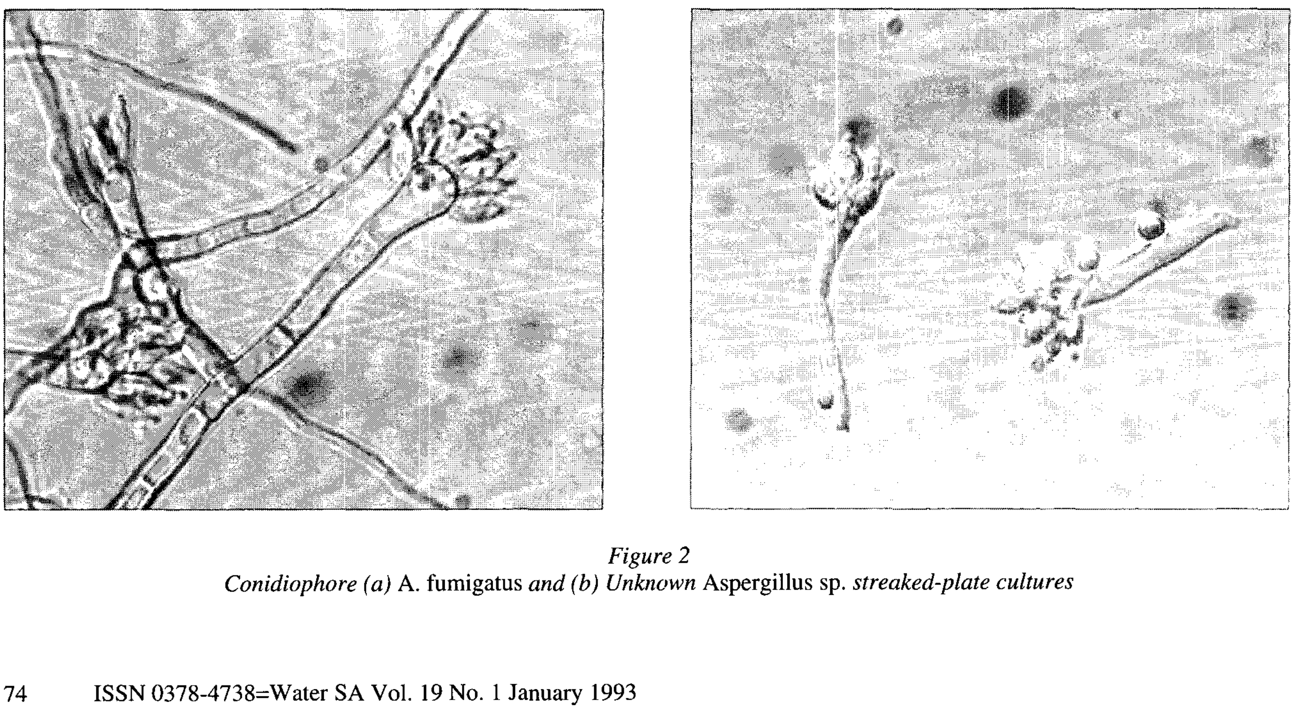

Figure 2
Conidiophore (a) A. fumigatus *and (b) Unknown* Aspergillus sp. *streaked-plate cultures*

TABLE 2
TYPICAL STEADY STATE DATA FOR THE CONTINUOUS CULTIVATION OF *ASPERGILLUS* SP. GROWN ON SSL AT 45°C

τ (h)	Θ_c (h)	S_o (g COD·ℓ$^{-1}$)	S (g COD·ℓ$^{-1}$)	X (g biomass·ℓ$^{-1}$)
2,65	4,46	2,829	0,568	2,93
2,52	5,42	2,482	0,922	2 71
2,88	6,24	2,705	0,894	2 94
2,71	8,15	2,856	1,115	3,61
2,76	9,15	2,989	0,509	5,39
2,52	11,51	3,239	1,221	5,32
2,52	13,18	2,914	1,628	5,76
3,28	14,93	3,542	1,190	4,53
3,33	16,95	3,528	1,151	5,92

τ = hydraulic residence time
Θ_c = cell residence time
S_0 = biodegradable feed concentration
S = effluent concentration
X = biomass concentration

diluted SSL of 10 g COD·ℓ$^{-1}$ as used here as substrate has a biodegradable concentration of only 3,36 g COD·ℓ$^{-1}$.

Kinetic constants

Typical steady state data are shown in Table 2.

The kinetic constants were calculated from the data in Table 2 by the methods of Grady and Lim (1980) and are shown in Table 3.

Crude protein concentration and amino acid profile of *A. fumigatus*

The results of the micro-Kjeldahl and amino acid analyser are shown in Table 4.

Discussion and conclusions

Although it was reported that *A. fumigatus* is a thermotolerant organism and could grow at 57°C (Domsch et al., 1980), sustainable growth could only be maintained at temperatures < 50°C, with a drastic drop in productivity at temperatures > 45°C (Fig. 1). Also, at temperatures < 45°C other species of *Aspergillus* started to dominate. These results show that although *A. fumigatus* could tolerate temperatures of up to 50°C, which would be beneficial for the treatment of high temperature effluents, optimum biomass production was only at 45°C and below. Thus for maximum biomass production and the selection pressure benefit of high temperatures a temperature of 45°C is recommended in open selective reactors.

Optimum pH was between 5,5 and 6 as shown in Fig. 2. As *Aspergillus* sp. grow relatively poorly at pH below 5,5, the suppression of bacterial growth due to low pH was not as significant as was observed with the selective cultivation of *Geotrichum* sp. (Kühn and Pretorius, 1989). To make the best use of pH as a selection factor (Pretorius, 1987) it is recommended that a pH of 5,5 be used.

The capability of *Aspergillus* sp. to utilise only 33,6% of the SSL-COD was in the same region as *Geotrichum candidum* which could reduce the SSL-COD by 38,2% (Lempert, 1992).

TABLE 3
SUMMARY OF KINETIC CONSTANTS OF *A. FUMIGATUS* GROWN ON SSL

Parameter	Unit	Value
pH		5,5
Temperature	°C	45
Maximum growth rate (μ_{max})	h^{-1}	0,318
Saturation constant (K_s)	mg COD·ℓ$^{-1}$	260
True yield (Y)	mg biomass (mg COD)$^{-1}$	0,70
Decay rate (b)	h^{-1}	0,016

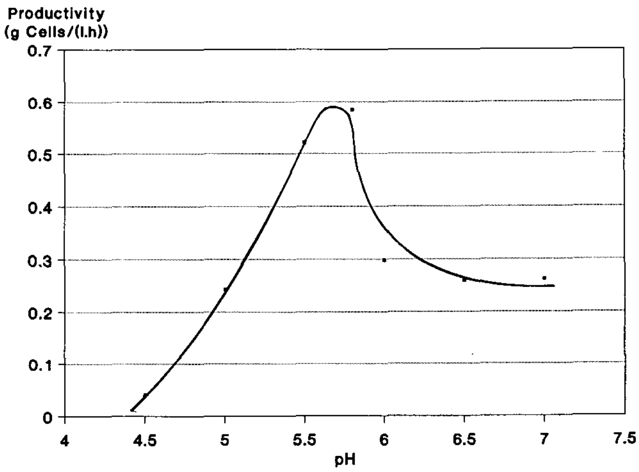

Figure 3
The effect of pH on the biomass production of A. fumigatus

TABLE 4
CRUDE PROTEIN AND AMINO ACID PROFILE OF *ASPERGILLUS* SP.

Amino acid	*Aspergillus sp.* g·(100 g)$^{-1}$
Alanine	2,8
Argenine	2,8
Aspartic acid	3,6
Cystine	ND
Glutamic acid	5,4
Glycine	2,1
Histidine	0,9
Isoleucine	1,9
Leucine	3,1
Lysine	2,6
Methionine	0,7
Phenylalanine	1,7
Proline	2,1
Serine	1,9
Threonine	1,6
Tyrosine	1,2
Valine	2,2
% N	7,9
* % Crude protein	49,4
% True protein	>36,5

ND - not determined
*Kjeldahl x 6,25

This relatively low SSL-COD reduction means that some additional secondary treatment should be considered for treating the effluent from an SCP production plant using SSL as carbon source.

The maximum growth rate (μ_{max}) of 0,318 h^{-1} and a yield coefficient (Y) of 0,7 (Table 3) compare favourably with a μ_{max} of 0,26 h^{-1} and a Y_g of 0,384 as reported for *G. candidum* grown on petrochemical effluents (Kühn and Pretorius, 1989). It is especially the relatively high Y that is of interest for SCP. In such a case the mass of oxygen required and the amount of biochemical heat generated are substantially less for the same mass of SCP produced than for micro-organisms with a lower yield coefficient (Bailey and Ollis, 1986).

The true protein content of ± 36,5% for *A. fumigatus* was significantly lower than the 45,5% observed for *G. candidum* (Kühn and Pretorius, 1989). The amino acid composition of *Aspergillus* sp. compares well with other protein sources generally used as feed for animals. The slightly higher concentrations of the essential amino acids lysine and methionine make the SCP of *Aspergillus* sp. also superior to SCP from *G. candidum* (Nell, 1992).

In conclusion it seems that the thermotolerant *A. fumigatus* should be seriously considered for the production of SCP on the SSL effluents of pulp mills. Although no conidiospore formation was ever observed in the liquid cultures, the SCP produced from *A. fumigatus* should be further investigated for any toxicological and pathogenic properties.

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