The selective cultivation of the thermotolerant *Aspergillus* sp. on spent sulphite liquor

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Abstract

Spent sulphite liquor (SSL) from a pulp mill was evaluated as a potential source for single-cell protein (SCP) production. The lowering of the relatively high discharge temperature of the effluent as well as the removal of biologically generated heat could hamper the economics of SCP production. In this paper a description is given of the dynamic selection of a thermotolerant *Aspergillus* sp. that proliferates on SSL at 45°C and which has the potential of SCP production at elevated temperatures.

Introduction

In South Africa the temperature of municipal waste water generally varies between 10°C and 30°C (Water Research Commission, 1984). With a chemical oxygen demand (COD) of 500 to 800 mg·ℓ^{-1}, the heat generated during the biological purification process is less than the heat lost due to aeration (Eckenfelder, 1980). Except for compensating during the design phase for the rate limiting effects of temperature on nitrification in biological nutrient removal plants, no special attention is given to temperature in municipal waste-water treatment plants.

In contrast to municipal waste water, many industrial effluents have both high COD concentrations and temperatures. Values reported for sugar mill effluents were 1,5 to 2,0 g COD·ℓ^{-1} at 45° to 70°C (SRK Inc., 1990a) and 18 to 26 g COD·ℓ^{-1} at 45° to 75°C for paper and pulp mill effluents respectively (SRK Inc., 1990b). These effluents should either be cooled to ambient temperatures and below for ordinary mesophilic biological treatment, or special care should be taken to operate in the thermophilic temperature range.

The growth of filamentous organisms in relatively dilute effluents at mesophilic temperatures has been investigated as a possible process for the production of single-cell protein (SCP) (Pretorius and Hensman, 1984; Kühn and Pretorius, 1988; Kühn and Pretorius, 1989a; b). A comprehensive cost analysis on the full-scale production of SCP from petroleum waste water has indicated that the initial cooling of the effluent and the subsequent removal of biologically generated heat constitute a major part of the production costs (De Wet, 1992). Attempts to isolate a thermophilic filamentous micro-organism which could use this effluent were unsuccessful.

Pulp and paper mill effluents have been identified as a possible source for the production of SCP (Cloete, 1990). The chemical composition of this effluent is quite different from the petrochemical effluent. Since this effluent is also discharged at a temperature above 45°C, producing SCP from this effluent is also subject to the cost of cooling. It would thus be advantageous if an easily cultivable and harvestable thermophilic filamentous micro-organism suitable for SCP could be found for this type of effluent. The purpose of this research was to selectively cultivate, identify and evaluate thermophilic filamentous micro-organisms capable of utilising a pulp mill effluent for possible SCP production.

Pulp mill effluent

One of the major pulp mills in South Africa uses approximately 85% *Eucalyptus* and 15% wattle wood and the calcium-based sulphite pulping process to produce cellulose pulp. In this pulping process about 275 000 t of a lignosulphonated black liquor (known as spent-sulphite liquor (SSL)) is produced annually (Sappi/Saiccor, 1991). Typical chemical analysis of the undiluted effluent is shown in Table 1.

At the pulp mill the concentrated SSL is diluted with water from the washing and bleaching processes to give a final effluent with an organic content of 16 to 28 g COD·ℓ^{-1} and a temperature of 45 to 60°C (SRK Inc., 1990b). This effluent is discharged at a rate of 80 000 $m^3 \cdot d^{-1}$ into the Indian Ocean (Sappi/Saiccor, 1991).

TABLE 1
CHEMICAL COMPOSITION OF SSL (JURGENSEN AND PATTON, 1979)

Constituent	% of solids	Typical concentration g·ℓ^{-1}
Total sugars	15 - 22	93
Volatile acids (as acetic)	2 - 5	0 - 8
Lignin (as lignosulphonates)	50 - 65	354
Sulphur (all forms as S)	8 - 10	43
COD		160 - 230
BOD_5		25 - 80

Materials and methods

Selection reactor

A continuously fed reactor equipped with a crossflow-microscreen as described by Kühn and Pretorius (1989b) and shown in Fig. 1 was used as reactor.

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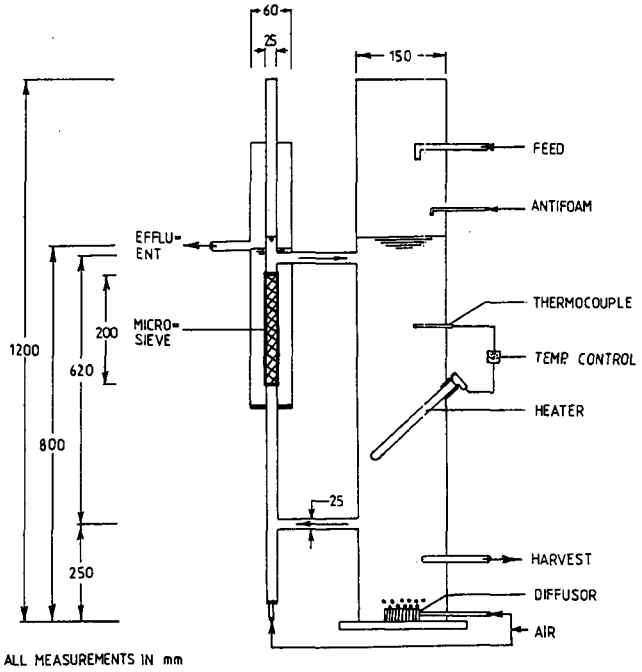

Figure 1
Experimental selector reactor

The temperature in the reactor could be controlled (± 0,2°C) at any desired temperature from room temperature to 70°C. The reactor was provided with the necessary feed supply and biomass harvesting pumps. The main laboratory compressed air supply was used for aeration.

Substrate

Diluted SSL effluent (COD between 16 and 28 $g.\ell^{-1}$) was collected and further diluted with tap water to a COD of 10 $g.\ell^{-1}$, which was equivalent to a biodegradable COD of approximately 4 000 $mg.\ell^{-1}$. This diluted SSL was supplemented with nutrients as described by Tabak and Cooke (1968) and the pH was adjusted to 5,5 with diluted H_2SO_4. The diluted SSL substrate (DSSL) was stored at 2°C. Fresh batches of DSSL were prepared weekly.

Initial inoculum and enrichment

To include the widest possible range of suitable micro-organisms, samples were collected from garden topsoil, garden compost heaps and various sewage samples.

Approximately 1 ℓ quantities of topsoil and compost respectively were covered with 2 ℓ tap water, stirred and allowed to settle for a few minutes. The supernatants were decanted and strained through a 1-mm opening size screen into the selector reactor. In addition 2 ℓ each of raw domestic sewage and mixed liquor collected from the aeration basin of an activated sludge plant were added to the selector reactor.

The reactor was filled to its working capacity (17,5 ℓ) with DSSL and to suppress unwanted bacterial growth, three chloramphenicol capsules (125 mg each) were added. The temperature was adjusted to 45 ± 0,2°C and aeration was commenced at a superficial rate of 0,5 $m{\cdot}min^{-1}$. This initial enrichment was maintained as a batch culture for 12 h.

Dynamic selection

After 12 h of static enrichment, feed and biomass harvesting was commenced according to an operating schedule shown in Table 2.

Once feed is commenced the combination of hydraulic residence time and microsieve size act as a dynamic selector for selecting mainly filamentous micro-organisms from the statically enriched culture (Pretorius and Hensman, 1984).

Identification of dominating species

Once a stable microbial population was obtained, samples of the reactor contents were streaked out on Rose Bengal (Difco) agar plates and incubated at 45°C. Pure cultures of the dominating species were identified according to Onions (1982).

Results

The chloramphenicol (which is a broad-spectrum antibiotic) suppressed the development of fast-growing bacteria and allowed the germination of fungal spores so that small fungal

TABLE 2
FEED AND BIOMASS HARVESTING SCHEDULE FOR DYNAMIC SELECTION

Time after start-up (h)	Hydraulic residence time τ* (h)	Biomass residence time Θ_c* (h)	Time at constant operation (h)
0 - 12	∞	∞	12
12 - 36	32	∞	24
36 - 66	24	∞	30
66 - 90	18	24	24
90 - 114	9	24	24
114- 138	6	18	24
> 138	3	Varying	-

$*\tau = \frac{\text{Reactor volume}}{\text{Flow rate}}$ $*\Theta_c = \frac{\text{Biomass in reactor}}{\text{Biomass harvested}{\cdot}d^{-1}}$

hyphae could be observed within 12 h after start-up.

Once feeding was commenced, smooth compact "sclerotic" type pellets (Metz and Kossen, 1977) varying from 1 to 5 mm in diameter (see Fig. 2) were starting to dominate the biomass. This type of fungal growth form dominated the biomass until biomass harvesting started about 66 h after initial start-up (Table 2), when the smooth, well-developed pellets changed to a pulp-like fungal mass as shown in Fig. 3.

This relatively homogeneous pulp-like fungal mass dominated the biomass upon further variations in τ and Θ_c.

Identification

The dominating fungal species was identified as *Aspergillus* sp. (Onions, 1982), possibly *Aspergillus fumigatus*.

Discussion and conclusion

A previous study (Kühn, 1989a; 1990) has shown that the fungus *Geotrichum candidum* could successfully be grown with the microsieve technique on SSL effluent from a pulp mill. For the commercial application of this process two major drawbacks were realised, namely the limited high temperature tolerance (max 37°C) of *G. candidum* and the difficulty of maintaining a monoculture due to the relative abundance of easily fermentable wood sugars. Since it was possible, as shown in this study, to selectively cultivate a fungus capable of growing at 45°C, most of the mentioned problems were overcome. The capability of this organism to grow at 45°C reduced the cost of cooling and the elevated temperature also exerted additional selection pressure (Pretorius, 1987) which limits the number of species.

The dominating fungal species was identified as most probably being *Aspergillus fumigatus*. According to Domsch et al. (1980), *A. fumigatus* is not a true thermophile but rather a thermotolerant fungus, since it can grow well at 20°C and can survive pasteurisation at 63°C for 25 min. This heat tolerance makes it an ideal organism for SCP production in a reactor where the temperature can fluctuate between 40 and 70°C.

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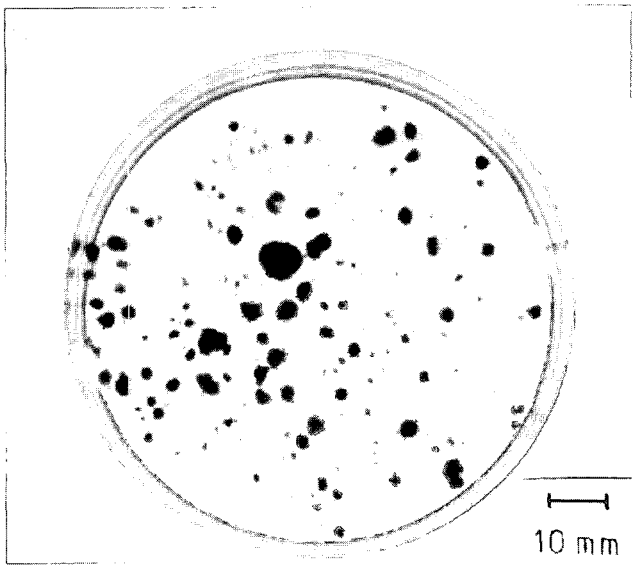

Figure 2
"Sclerotic" pellets dominating initial dynamic selection

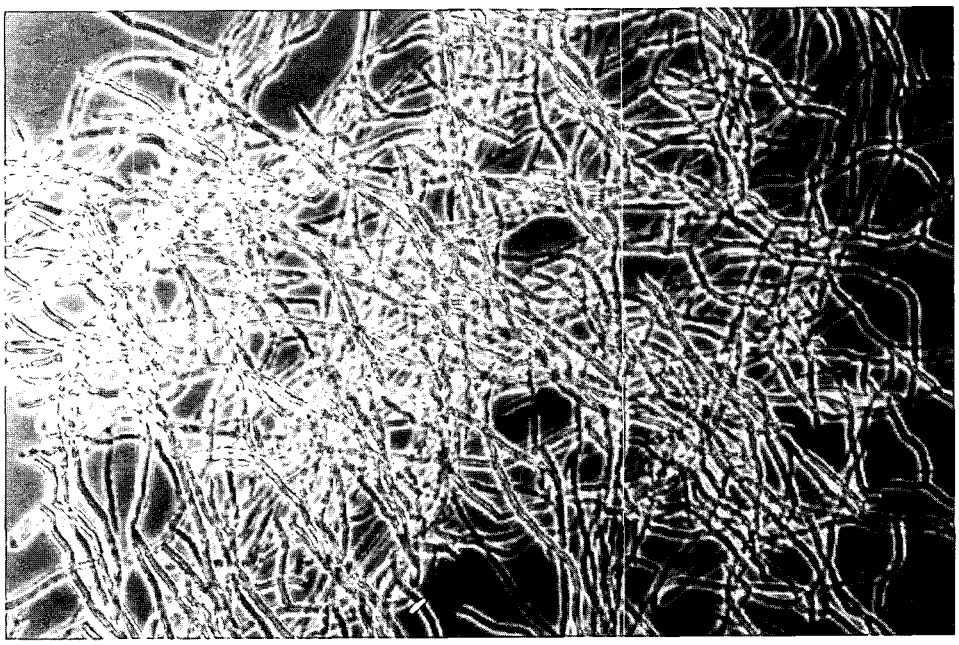

Figure 3
Pulp-like fungal mass

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