Mycoprotein Production on Spent Sulphite Liquor

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Report to the Water Research Commission

by the

Division of Water Utilisation Engineering
University of Pretoria

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MYCOPROTEIN PRODUCTION

ON

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EXECUTIVE SUMMARY

BACKGROUND

South Africa will have to augment its agriculturally produced protein to feed its ever increasing population. Since a global shortfall of protein can be expected to occur early in the next century, single-cell protein (SCP) production should be considered to relief future food shortages.

RESEARCH

Various high-volume effluent streams were identified as a potential source for single-cell protein (SCP) production to augment agriculturally produced protein in South Africa.

Spent sulphite liquor (SSL), the effluent from a pulp mill which utilises the calcium-based sulphite pulping process, was selected as a high-strength organic waste stream that can be utilised as a substrate for SCP production. The biodegradable COD of the pulp-mill's final effluent was found to be approximately 40% of the total COD, which translates into 234 000 $t.a^{-1}$ COD that will be available as substrate COD for SCP production.

The microsieve process was identified as the most suitable process for large-scale SCP production under continuous, non-aseptic culture conditions. Previous research (Kühn and Pretorius, 1989b) showed that continuous selective cultivation of the fungus *Geotrichum* sp. utilising SSL as substrate was possible with the microsieve process.

The microsieve process as operated at that stage revealed three major drawbacks for large-scale commercial application:

- Mesophilic fungi were employed that require extensive cooling to maintain optimum growth temperatures.

- It is difficult to maintain a monoculture due to excessive contamination by bacteria and yeasts at the low operating temperatures.

- Dilution of high substrate concentrations is needed to prevent oxygen from becoming the limiting substrate.

An alternative organism and/or process was therefore sought to continuously produce SCP at thermophilic temperatures.

The thermotolerant fungus, *Aspergillus fumigatus*, was dynamically selected. *A. fumigatus* proliferates on SSL at temperatures below 50°C and shows optimum growth near 45°C with an optimum pH between 5,25 and 5,75 when grown on SSL.

Growth kinetic constants for *A. fumigatus* were determined in a laboratory study at 45°C and a pH of 5,5. The following values were obtained:

- Biodegradability of SSL = 33,6%
- Maximum growth rate, μ_m = 0,318 h^{-1}
- Saturation constant, K_s = 260 mg COD.l^{-1}
- True growth yield, Y_g = 0,70 g cells.g COD^{-1} utilised
- Biomass decay rate, b = 0,016 h^{-1}

The amino-acid composition of *A. fumigatus* compares well with other protein sources generally used as feed for animals. A slightly higher concentration of the essential amino acids lysine and methionine renders the SCP from this fungus superior to SCP from *Geotrichum* sp.

It was possible to grow *A. fumigatus* continuously as a submerged culture in three different reactor configurations, *viz* a continuously stirred tank reactor (CSTR) with cell recycle, a CSTR without cell recycle and a series combination

of these two configurations. They were called Selector, Producer and Selector/Producer in series configuration, respectively. Monod kinetics were tested to describe reactor performance. It was found that theoretically predicted reactor performance resembled actual performance in a laboratory set-up.

Stable monoculture growth in non-aseptic conditions could only be maintained in a Selector and in a Selector/Producer in series reactor configuration. System behavior studies of the latter two configurations revealed that a Selector/Producer in series reactor required the least feed dilution, rendering this set-up the most economically viable process for large-scale SCP production.

System behavior for a Selector and Selector/Producer in series reactor configuration was studied and optimum performance of the two configurations was determined. Optimum oxygen utilisation required dilution of the feed in both the microsieve and the Selector/Producer configurations. With the latter configuration 75% less dilution was needed. Biomass production was 4% lower in the Selector/Producer reactor set-up than in the microsieve reactor.

Maximum theoretical SCP production utilising the pulp-mill effluent as substrate (flow = 80 000 $m^3.d^{-1}$; biodegradable COD = 6,8 $g.l^{-1}$) and *A. fumigatus* as fungus can be obtained in a microsieve process and would total approximately

120 000 t per annum. This figure represents 30% of South Africa's estimated total feed protein deficit in the year 2000.

FURTHER RESEARCH

The study that was undertaken under the heading *Mycoprotein production on spent sulphite liquor* opened up a completely new field for research, namely the continuous growth of fungi at thermophilic temperatures as fully submerged cultures. This research can be used for:

- Mycotoxigenic studies. Extensive feeding, toxicity and mutability studies with *A. fumigatus* should be undertaken to ensure its safe use as SCP.

- Pilot studies. A pilot plant for SCP production with the proposed process at thermophilic temperatures and using the fungus *A. fumigatus* is required to determine whether further development into a full-scale production process is justifiable.

- Production of microbial by-products. The use of *A. fumigatus* for the possible production of valuable by-products should be investigated.

- Mycoprotein production at elevated temperatures. Organic effluent that is discharged in large quantities at an elevated temperature by other "wet" industries in South Africa can be assessed for its value as a substrate to produce SCP.

- Biological effluent treatment at elevated temperatures. Maximising the COD removal from biological effluents at elevated temperatures instead of mycoprotein production could be investigated. Effluent treatment was not considered in this study.

BESTUURSOPSOMMING

AGTERGROND

Indien Suid Afrika in die toekoms selfonderhoudend ten opsigte van proteïen wil wees, sal die land sy beperkte landbouproteïene-produksie moet aanvul om in die groeiende bevolking se behoeftes te voorsien. Aangesien daar al vroeg in die volgende eeu met 'n wereldwye tekort aan proteïen gereken kan word, sal enkelselproteïen(ESP)-produksie oorweeg moet word om toekomstige voedsel tekorte te verlig.

NAVORSING

Verskeie hoë-volume, organiese uitvloeisels is geïdentifiseer as 'n moontlike bron vir ESP-produksie in Suid Afrika ter aanvulling van proteïene wat deur die landbou geproduseer word.

Sulfietloogresvloeistof (SLRV), die uitvloeisel van 'n spesifieke pulpmeule wat die kalsium-bisulfiet proses gebruik, is geïdentifiseer as 'n sterk organiese uitvloeisel wat gebruik kan word as substraat vir ESP-produksie. Daar is gevind

dat die fraksie biologies afbreekbare CSB van die finale uitvloeisel van die pulpmeule naastenby 40% van die totale CSB uitmaak. Hierdie persentasie verteenwoordig 234 000 ton CSB wat jaarliks beskikbaar is as substraat CSB vir ESP-produksie.

Die mikrosif-proses kon geïdentifiseer word as die mees geskikte proses *huidiglik beskikbaar vir grootskaalse ESP-produksie*. *Vorige navorsers* (Kühn en Pretorius, 1989b) het aangetoon dat kontinue, selektiewe kweking van die fungus *Geotrichum* sp., met SLRV as substraat, in die mesofiele temperatuurgebied moontlik was met behulp van die mikrosif-tegniek.

Vir grootskaalse, kommersiële aanwending van die mikrosif-proses vir ESP-produksie was daar egter nog verskeie struikelblokke:

- Die mesofiele fungi wat gebruik was vereis kunsmatige verkoeling om optimum bedryfstemperature to handhaaf. Kunsmatige verkoeling verhoog die kapitaal- en bedryfskoste van volskaalse prosesse en maak ESP-*produksie onekonomies*.

- Dit is moeilik om 'n monokultuur te handhaaf (binne die reaktor) as gevolg van buitensporige kontaminasie deur bakterië en giste by bedryfstemperature in die mesofiele gebied.

- Verdunning van die substraat is nodig ten einde te verhoed dat suurstof die groeibeperkende substraat word.

Om bostaande tekortkominge te oorkom, is daar na 'n alternatiewe organisme (fungi) en/of proses gesoek wat in die termofiele temperatuurgebied bedryf kan word.

'n Termotolerante fungus, *Aspergillus fumigatus*, wat SLRV kan metaboliseer en by temperature tot onder 50°C kan groei, kon dinamies geselekteer word. Daar is gevind dat hierdie fungus optimaal by 'n temperatuur van 45°C en 'n pH tussen 5,25 en 5,75 groei.

Die groeikonstantes van *A. fumigatus* met verdunde SLRV as voer by 'n temperatuur van 45°C en 'n pH van 5,5 was as volg:

- Biologies afbreekbare fraksie van CSB = 33,6%
- Maksimum spesifieke groeitempo, μ_m = 0,318 h^{-1}
- Versadigingskonstante, K_s = 260 mg CSB.l^{-1}
- Waargenome selopbrengskoëffisiënt, Y_g = 0,70 g selle.g CSB^{-1} gebruik
- Spesifieke biomassa verliestempo, b = 0,016 h^{-1}

Die aminosuurprofiel van *A. fumigatus* vergelyk goed met ander proteïen-bronne wat algemeen as voer gebruik word. In vergelyking met *Geotrichum* sp. besit *A. fumigatus* 'n effe hoër konsentrasie van die aminosuure lisien en metionien wat laasgenoemde fungus meer waardevoller as ESP maak.

A. fumigatus kon kontinu in 'n waterige medium met drie verskillende reaktoropstellings, nl 'n konstant-stromend, geroerde tenkreaktor (KSGTR) met selhersirkulasie, 'n KSGTR sonder selhersirkulasie en 'n kombinasie van hierdie twee reaktore in series, gekweek word. Die reaktore is onderskeidelik 'n Selektor, Produseerder en 'n Selektor/Produseerder in series konfigurasie genoem. Monod-groeikinetika kon gebruik word om die werkverrigting van die reaktore te beskryf. Teoretiese en werklike data het goed ooreengestem vir die eksperimentele reaktoropstelling.

'n Stabiele monokultuur van A. *fumigatus* kon slegs onder nie-aseptiese toestande in 'n Selektor en in 'n Selektor/Produseerder in series reaktor konfigurasie gehandhaaf word. Die Selektor/Produseerder in series reaktore vereis, van die verskillende opstellings wat geëvalueer is, die minste verdunning van die SLRV en maak dit dus die mees ekonomiese proses vir volskaalse ESP-produksie.

Optimum bedryfstoestande vir 'n Selektor en in 'n Selektor/Produseerder in series reaktor konfigurasie is ondersoek. Vir optimale benutting van suurstof moet die substraat (voer) na beide opstellings verdun word. Die Selektor/Produseerder in series reaktore benodig ongeveer 75% minder verdunningswater en lewer ongeveer 4% minder biomassa as 'n Selektor reaktor.

Die maksimum teoretiese hoeveelheid ESP wat geproduseer kan word indien die uitvloeisel (vloei = 80 000 m^3d^{-1}; bioafbreekbare CSB = 6,8 $g.l^{-1}$) van die pulpmeule gebruik word as substraat om *A. fumigatus* te kweek is ongeveer 120 000 t per jaar. Hierdie hoeveelheid verteenwoordig 30% van Suid Afrika se totale beraamde voerproteïen tekort vir die jaar 2000.

VERDERE NAVORSING

'n Totaal nuwe studieveld is tydens hierdie ondersoek geopen, nl die kontinue kweking van fungi in die termofiele temperatuurgebied as volledig ondergedompelde kultuur. Moontlike verdere navorsing in hierdie veld sluit in:

- Mutageniteitstudies. Uitgebreide voeding-, toksisiteit- en mutageniteitstudies moet onderneem word ten einde te verseker dat *A. fumigatus* veilig as ESP benut kan word.

- Loodsaanleg. Toetse met 'n aanleg op loodskaal, wat ESP-produksie by termofiele temperatuure (met *A. fumigatus* as fungus) ondersoek, word benodig om te bepaal of die voorgestelde prosesse potensiaal toon vir volskaalse ESP-produksie.

- Produksie van mikrobiese by-produkte. Die moontlike gebruik van *A. fumigatus* om waardevolle by-produkte te lewer, kan ondersoek word.

- ESP produksie by hoër temperature. Organiese uitvloeisels wat in groot hoeveelhede by hoër as omgewingstemperature gestort word, se potensiaal as substraat vir ESP-produksie kan bepaal word.

- Biologiese uitvloeisel suiwering by hoër temperature. Bedryfsparameters om maksimale CSB-verwydering by hoër as omgewingstemperature te bewerkstellig, in stede van biomassaproduksie, kan ondersoek word. Tydens hierdie studie is die aspek van uitvloeiselbehandeling nie aangespreek nie.

CONTENTS

CHAPTER V: BIOMASS PRODUCTION OF *ASPERGILLUS FUMIGATUS* ON SPENT SULPHITE LIQUOR IN NON-ASEPTIC CULTURE CONDITIONS

NOTATION

Symbol	Meaning	Units
a	Bubble surface area per unit volume	
AO	Oxygen transfer rate	g O_2.(l.h)$^{-1}$
b	Specific biomass decay rate	h^{-1}
BOD	Biochemical oxygen demand	g.l^{-1}
c*	Oxygen solubility in a specific liquid	
c	Dissolved oxygen concentration in a specific liquid	
COD	Chemical oxygen demand	g.l^{-1}
CSTR	Continuous stirred tank reactor	
DSSL	Diluted spent sulphite liquor	
F	Volumetric flow rate	m^3.h^{-1}
G_S	Volumetric air feed rate	
H, H_c, H_S	Heat of combustion with c and s denoting cell material and substrate respectively	kcal.g cells^{-1}
k_l	Mass transfer coefficient	
K_S	Saturation constant	g COD.l^{-1}
M_O	Specific weight of oxygen in air	
OA	Oxygen available (theoretical value)	g O_2.(l.h)$^{-1}$
P	Biomass production rate	g cells.(l.h)$^{-1}$
q	Specific substrate removal rate	g COD.(h.g)$^{-1}$
r_o	Oxygen utilisation rate	g O_2.(l.h)$^{-1}$
R	Mass transfer rate	
RO_t	Quantity of oxygen utilised (V. r_o)	g O_2.h^{-1}
S	Soluble COD in the reactor	g COD.l^{-1}
S_O	Soluble COD in the inflow (feed)	g COD.l^{-1}
S_1, S_2	Soluble COD in the respective reactor (eg 1 or 2)	g COD.l^{-1}
SCP	Single-cell protein	
SS	Suspended solids	g.l^{-1}
SSL	Spent sulphite liquor	
V	Volume of reactor	m^3
X	Cell- or biomass concentration	g.l^{-1}

Y	Observed growth yield	$g.g\ COD^{-1}$
Y_g	True growth yield	$gcells.gCOD^{-1}$
ß	Oxygen demand per unit mass of cells	$g\ O_2.g$ cells
δ	Specific bacterial death rate	h^{-1}
Δ	Difference	
ε	Oxygen transfer efficiency	
θ	Reactor space time	h
θ_c	Mean biomass retention time	h
τ_h	Mean hydraulic retention time	h
τ_1, τ_2	Mean hydraulic retention time referring to a specific reactor, eg no 1 or 2	h
μ	Specific biomass growth rate	h^{-1}
μ_m	Maximum specific biomass growth rate	h^{-1}

CHAPTER I

BACKGROUND

South Africa may be considered an arid country with an average annual rainfall of only 502 mm, which is far below the world average of 860 mm (Department of Water Affairs, 1986). In spite of the relatively low rainfall and poor agricultural climatic conditions, South Africa is one of only a few countries on the African continent that is generally self-sustaining and, in average to good rainfall seasons, a net exporter of food (Cloete, 1990). This occurs against a background of sustained imports of protein-rich materials expressed as fish meal and oilcake equivalents. In the 1992/93 financial year, a total of 192 000 t of protein were imported (ARC, 1993). At a cost of R1 790 per ton of imported fish meal and R1 030 per ton of oilcake (Swart, 1993) this amounts to approximately R450 m.

The high population growth and expected general upliftment of the people of South Africa will result in ever increasing animal protein consumption, necessitating higher protein imports in future. Du Preez (1990) states that South Africa, with a 3,1% population growth rate, had the highest average annual population growth rate in the world after the Arab countries. If this population growth rate is maintained, Cloete (1990) estimated that the total feed protein deficit by the year 2000 will be approximately 400 000 t.

Recognition of the increasing and sustained protein deficit led to the establishment of the Protein Advisory Committee (PAC) in 1976 (Cloete, 1990). It had to monitor the short and long-term requirements for feed proteins, to optimise utilisation of the available sources and to develop new sources. The PAC recommended a two-pronged

approach, namely that agricultural protein production be encouraged and alternative protein sources be developed. Agricultural protein production could be enhanced by encouraging the cultivation of high-protein crops such as soybeans, lupines, high-lysine yellow maize, triticale and legume pastures (Du Preez, 1990). Alternative protein sources include the recovery of waste proteins from plant and animal-processing plants, exploiting alternative marine sources, and the possible production of non-conventional proteins like single-cell proteins (SCP) from certain identified industrial effluents.

Though protein production by the agricultural sector can be increased considerably with more scientific and intensive production methods, Du Preez (1990) and Cloete (1990) doubt that it can be substantially increased because of the country's inconsistent rainfall and unfavourable climatic conditions. This necessitates the investigation and exploitation of alternative protein sources. The possible non-conventional production of protein from organic pollutants in industrial waste water forms the subject of this investigation.

CHAPTER II

NON-CONVENTIONAL PROTEIN PRODUCTION

1. INTRODUCTION

Cells from algae, bacteria, fungi and yeasts, produced to substitute or augment the protein contents in human and animal diets, fall under non-conventional proteins and are generally known as "single-cell proteins" (Lipinsky & Litchfield, 1974). Initially the term "single-cell protein" was appropriate because unicellular organisms like yeasts were used to produce microbial biomass for food and food products (Turnbull, 1982). Nowadays single and multicellular microorganisms are mass cultivated for this purpose (Litchfield, 1977). Though "cultured microbial biomass" would therefore be a more appropriate term (Du Preez, 1990), the term "single-cell protein" (SCP) has generally been accepted in the scientific community (Kihlberg, 1972) and, to avoid confusion, will also be used here.

Interest in SCP production has existed since the turn of the century (Solomons, 1983). Food shortages experienced during and after World Wars I and II caused several governments to finance projects to produce microbial protein for human and animal nutrition, mainly during and after these wars. Later the production of a cheap alternative for meat and animal feed ingredients seemed

possible and attracted renewed interest in SCP production (Lipinsky & Litchfield, 1974). This interest was further encouraged by estimates of future protein demands. Hoshiai (1981), for example, predicted a global shortfall of 14 million tons of feed protein by the year 2000.

Many studies were undertaken and numerous experimental, pilot and full-scale SCP production processes were developed. The different substrates, processes and microorganisms employed are summarised in Appendix A (Table A-1). However, there are no large-scale plants ($\geq$ 10 000 $t.a^{-1}$) to date that produce SCP in any country where a free-market economy prevails (Du Preez, 1990), the reason being that many problems were encountered that rendered full-scale SCP production not economically viable.

2. PROBLEMS ENCOUNTERED WITH SCP PRODUCTION

The production of SCP is a microbiological process to cultivate microorganisms in bioreactors (fermentors). Commercial SCP processes are capital-intensive, with relatively high costs for raw materials and energy and generally low labour costs (Reed, 1982). Moo-Young (1977) made a study of the cost of commercial SCP production and broadly divided the various cost items as follows:

- Raw materials
  - Substrate — 17% to 64%
  - Nutrients and miscellaneous — 13% to 38%
- Utilities — 12% to 37%
- Labour — 5% to 11%
- Depreciation — 6% to 10% per annum

The selective importance of these cost items is further elucidated.

2.1 Cost of raw materials

The substrate constitutes a major portion of the total raw material costs required to produce SCP. To reduce these costs, various and, where possible, cheap raw materials have been used as a substrate for SCP production. Cheap petroleum compounds like n-alkanes (C_{12} - C_{18}) were used as substrate in the 'British Petroleum' process to produce different yeasts (Atkinson & Mavituna, 1983), methane gas was used in the 'Shell' process to grow bacteria (Bailey & Ollis, 1986), and methanol was used in the 'Pruteen' process to produce SCP in the form of bacteria (Lipinsky & Litchfield, 1974).

Relatively expensive glucose was recently used in the 'Quorn' process to grow the fungus *Fusarium graminearum* (Du Preez, 1990). In this case the use of glucose is justified, as the product is not sold as a cheap substitute for meat, but priced similarly to prime cuts.

Substrate costs can be expected to vary considerably, depending on the type and availability of the substrate. Litchfield (1977) suggests that the figure for hydrocarbons such as methanol and ethanol lies between 45% and 60% of the total operating costs. Moo-Young *et al.* (1980) calculated the substrate costs for methanol-based processes, excluding nutrients and other chemicals, to be 37,6% of the total production costs. This figure increased to 61,6% when molasses was used as a substrate for SCP production.

Although Quorn was able to penetrate the human food market, despite being expensive compared to other protein sources, the same would not apply when the animal fodder market is targeted (Solomons, 1983). SCP processes that utilise waste streams with high organic loads as substrate are therefore more likely to succeed in providing a low-cost alternative protein-rich fodder for animals. The reduced organic material in the waste stream due to SCP production may also result in additional savings because less or no additional biological treatment and/or penalties may be necessary to dispose of the effluent stream. Developing an SCP process based on waste water as substrate was emphasised during this research.

2.2 Energy costs

Heat removal and oxygen transfer constitute the major energy costs for fermentation (Chen *et al.*, 1987; Mateles, 1975; Reed, 1982). From figures presented by Abbott and Clamen (1973) it was observed that heat removal for various SCP processes varies between 6% and 74%, and oxygen transfer between 3% and 39% of the total operating costs. The calculated average costs for heat removal and oxygen transfer were 33% and 17% respectively. Any savings here could contribute towards establishing an economically viable SCP process.

Heat removal. Optimal growth of microorganisms requires specific culture conditions of which temperature is an important one (Litchfield, 1977). The temperature must be kept between a relatively narrow upper and lower limit for a specific microorganism to grow in optimum conditions. Large-scale SCP production processes have utilised mesophilic microorganisms such as yeast, bacteria and algae, with optimum operating temperatures between 28° and 32°C, 28° and 38°C, and 30° and 40°C respectively (Kihlberg, 1972).

Heat, which mainly stems from three activities, has to be removed to maintain optimum growth conditions:

- Sterilisation. Conventional fermentation processes rely mostly on heat sterilisation of the feed to maintain aseptic growth conditions (Atkinson & Mavituna, 1983; Solomons, 1983). This is typically achieved at elevated temperatures (15 min at 121°C). Subsequent cooling of the feed to optimum operating temperatures is then necessary.

- Industrial effluents. Many industrial effluents are discharged at elevated temperatures. Sugar-mill effluents are discharged at 45° to 70°C (Steffen *et al.*, 1990b), synthetic fuel effluents at 40° to 70°C (De Wet, 1992), and pulp-mill effluents at 45° to 75°C (Steffen *et al.*, 1990a). Cooling is necessary if one of these waste streams is to be used as substrate for SCP production in the mesophilic temperature range.

- Microbial activity. Heat is generated during growth due to the thermodynamics of overall microbial activity, which is related to the utilisation of the carbon and energy source. The amount of heat released during SCP production largely depends on cell yield. The yield factor is not a constant for a particular organism but depends on optimum growth conditions in terms of pH, temperature, culture medium, oxygen tension and the substrate used (Solomons, 1983).

  According to Atkinson and Mavituna (1983), only 40% to 50% of the available enthalpy in the substrate is conserved in the biomass when the carbon source is utilised for biomass production through anabolism during growth. The rest of the enthalpy is released as heat.

The approximate amount of heat generated when a specific substrate is biologically degraded can be estimated from energy balances using simplified metabolic pathways for biological substrate consumption. According to Abbot and Clamen (1973), heat evolution (in kcal.g cells^{-1}), ΔH, can be approximated by the following equation:

$$\Delta H = \frac{\Delta H_s - Y_g . \Delta H_c}{Y_g} \quad \ldots\ldots (1)$$

where ΔH_s and ΔH_c represent the heats of combustion (in kcal.g cells^{-1}) of the substrate and cell material respectively and Y_g represents the experimentally obtained growth yield (g cells.g COD^{-1} utilised).

From Equation (1) follows that, if cell yields do not change, more heat will be produced when more reduced compounds such as hydrocarbons are employed as substrate instead of partially oxidised compounds, because $\Delta H(CH_4) > \Delta H(CH_3OH)$ and ΔH(n-alkanes)$>\Delta H$(glucose). Using a more reduced substrate will therefore require more extensive cooling equipment to maintain optimum temperature conditions.

Since heat removal equipment increases the capital outlay and operating costs, a SCP production process that could be operated at temperatures that exceed the ambient temperatures and that utilises an already partially oxidised substrate would be beneficial in terms of the cooling economy of the process.

Oxygen transfer. A common oxidant for aerobic growth is oxygen which is usually supplied by introducing air into the culture medium. The theoretical oxygen requirements to produce SCP from a carbon source can be estimated by

using an overall stoichiometric equation that converts the substrate to dry cells. The actual amount of air that has to be introduced into the fermentor to prevent oxygen from becoming the limiting substrate is generally higher than the theoretical amount and largely depends on the oxygen transfer from the air into the liquid phase.

Oxygen transfer in bioreactors is affected by the viscosity of the broth, the morphology and biomass concentration of the microbial species present, and the mechanical equipment employed (Atkinson & Mavituna, 1983).

- Effect of viscosity on oxygen transfer. Extensive research (Chen, 1990; Chain *et al.*, 1966; Phillips & Johnson, 1961) has shown that an increase in the viscosity of a broth leads to reduced oxygen transfer rates. Experiments with a sucrose solution (Solomons & Perkin, 1958) showed oxygen transfer rates to decrease by a factor of ten when the viscosity was increased from 1 cP (water) to 10 cP (45% sucrose). Similar observations were made with mould suspensions in fermentors.

  The reason for the reduced oxygen transfer rates observed in mycelial (mould) suspensions is a change in the flow characteristics of the suspension. At biomass concentrations of 2 g.cells l^{-1} and higher, mycelial broths are viscous, non-Newtonian fluids, and some mycelial suspensions even exhibit properties of Bingham plastics which adversely affect oxygen transfer (Moo-Young *et al.*, 1987). Wille (1992) established, for an airlift type of reactor, a relationship between the oxygen transfer, AO, and biomass concentration, X, of a filamentous microorganism, *Geotrichum candidum*, in a cross-flow microscreen reactor. This is given by:

$$AO = 1{,}491.\ e^{(-0{,}1591.X)}\ \ g\ O_2.(l.h)^{-1} \qquad \ldots\ (2)$$

and was found to apply to biomass concentrations of between 2 and 10 g cells.l^{-1}.

- Effect of microorganisms on oxygen transfer. Phillips and Johnson (1961) have shown that species with a more complex morphology (like filaments) lead to lower oxygen transfer rates because their presence causes an increase in the viscosity of the broth. They found, for example, that the oxygen transfer rate in a microbial suspension containing 12 $g.l^{-1}$ of the fungus *Aspergillus niger* was approximately a quarter that of the single-cell bacterium *Escherichia coli* at similar concentration and physical conditions.

- Effect of mechanical equipment on oxygen transfer. The efficiency of aeration systems varies considerably and depends on the degree to which the equipment can diffuse air into the liquid phase. The overall volumetric mass transfer rate, R, which is also used to describe the oxygen transfer from the gas to the liquid phase, is expressed by the following relationship (Atkinson & Mavituna, 1983):

$$R = k_l a\ (c^* - c) \qquad \ldots\ (3)$$

where k_l is the mass transfer coefficient, based on the liquid-phase resistance to mass transfer, a is the bubble surface area per unit volume, and c^* and c are respectively the oxygen solubility and the dissolved oxygen concentrations in the liquid.

The ratio of oxygen transferred to the liquid phase from the oxygen contained in the air, if the latter is blown into the liquid, is measured by the oxygen transfer efficiency, ε. This parameter is a function of the diffusion efficiency:

$$\varepsilon = \frac{k_l a.c^*.V}{M_O.G_S} \qquad \ldots\ldots (4)$$

where G_S represents the volumetric air-feed rate, V the volume of the fermentor, and M_O is the specific weight of oxygen in air (Kasakura *et al.*, 1990).

ε is generally increased by employing equipment that improves the diffusion of air (or gas) into the liquid phase. This parameter is affected by many factors, for example the shape and internal arrangements of the tank, the type of air diffuser system and the means of diffusion and the air flow rate, to name but a few (Atkinson & Mavituna, 1983). These researchers found that the oxygen transfer rates in a countinuously stirred tank increased threefold, from 30 to 120 mMol $O_2.(l.h)^{-1}$ when baffles were included. Kasakura *et al.* (1990) observed a 30% higher ε when diffusers with a pore diameter of 260 μm instead of 400 μm were employed in activated sludge plants. Reed (1982) found oxygen transfer efficiencies in yeast fermentors to improve from 20% to 50% due to proper mechanical dispersion of the incoming air by a larger, differently shaped impeller, higher air flow rates and higher impeller speeds. He concluded that, since oxygen is usually the limiting factor in achieving high productivity, the oxygen supply equipment is the most critical cost factor in the construction of fermentors.

If a specific reactor and air supply system are used to grow a specific microorganism, oxygen transfer will be affected by the biomass concentration inside the reactor, in accordance with Equation (2). To establish growth kinetic parameters for the fungus used in this study, the operating parameters for the experimental reactors were chosen to prevent oxygen from becoming the limiting substrate.

2.3 Harvesting and dewatering

Separation of the biomass from the culture medium is generally difficult to achieve and often requires sophisticated and costly equipment. The relative dilute nature of broths and effluents used for SCP production processes also requires that large volumes of water have to be handled. Typical concentrations for algal biomass are 1 to 2 $g.l^{-1}$ (dry weight) and for bacterial, yeast and fungal biomass 5 to 20 $g.l^{-1}$ (dry weight) (Atkinson & Mavituna, 1983). Various attempts have been made to increase cell concentration in the culture broth, *inter alia* to render centrifugation more economical. Gold *et al.* (1981) employed a cell recycle process, and Du Preez (1990) increased the substrate concentration by supplementing the feed with an additional carbon source.

Processes employed to recover the biomass include:

- Chemical-physical processes such as sedimentation and/or flotation with or without prior flocculation and electrocoagulation. These processes are mainly used to recover algal biomass because of their low concentrations in the culture medium (Litchfield, 1977). When a flocculant such as aluminium sulphate, ferric chloride, calcium hydroxide or a polymer is used, sedimentation usually improves. A disadvantage is that the SCP is contaminated with the flocculant.

- Mechanical processes. Bacteria and yeasts are small (0,5 to 2 µm and 4 to 8 µm respectively) and have a specific density (1,003 and 1,04 to 1,09 respectively) close to water (1,000) (Kihlberg, 1972). Expensive high-speed centrifuges are required for continuous separation and this increases capital outlay and can contribute as much as 16% towards product costs (Moo-Young, 1977). The cost of centrifugation could be four times higher for bacteria than for yeasts because of the smaller size of the bacteria. In contrast, fungi can be recovered relatively easily because of their bigger size (>10 µm) and filamentous nature, using basket centrifuges, rotary vacuum filters or medium to fine screens (Bailey & Ollis, 1986).

From the above follows that yeasts, bacteria and fungi can be cultivated at higher biomass concentrations, which requires less extensive process equipment for the same quantity of biomass produced. Filamentous fungi, compared to yeasts and bacteria, are easy to harvest and less expensive equipment is required to separate the biomass from the culture medium. An SCP process based on cultivating a filamentous fungus would therefore be economically more attractive than one which relies on bacteria or yeasts.

2.4 Miscellaneous

2.4.1 Toxic substances. Many waste streams used for SCP production contain substances which inhibit microbial growth. Heavy metals are generally toxic to microorganisms and can only be tolerated in low concentrations (Goodwin & Mercer, 1986). In pulp and paper-mill effluents sulphur dioxide inhibits yeast growth (Andersen, 1979), and all chemicals used for bleaching are highly toxic to most microorganisms (Water Research Commission, 1990). Furfural, found in sugar-mill effluent and

bagasse, inhibits yeast growth (Du Preez, 1990). Expensive pretreatment such as flocculation, ion exchange or stripping is often required to remove toxic substances from a specific stream before it can be used as a substrate for SCP production.

Residual toxic substances from the substrate, toxins produced by the microorganisms or pathogens contaminating the SCP product, may render the SCP produced unsuitable for human or animal consumption. SCP produced from substrates containing crude-oil fractions or *n*-paraffin may contain 3,4-benzyprene, methylcholanthrene and 1,2,5,6-dibenzanthracene which are highly carcinogenic (Kihlberg, 1972). A wide variety of potentially harmful mycotoxins such as aflatoxin, cholera toxin and tremorgen toxin, to name but a few, are produced by microorganisms. To remove these toxic substances often requires capital-intensive processes such as heat sterilisation, gas stripping, distillation and absorption, with subsequent cooling or extraction processes using costly solvents.

The spores of some bacteria and fungi pose a potential health hazard for humans and animals because they are primary pathogens. Clarck *et al.* (1984) found that personnel at wastewater treatment sludge compost operations who are exposed to high counts of fungal spores of particularly *Aspergillus* sp., notably *A. fumigatus, A. flavus, A. carneus* and *A. niger*, as well as asthma sufferers and immuno-suppressed patients are at risk of picking up aspergilloses. The disease known as 'farmers lung', which frequently occurred among mushroom compost workers, was ascribed to the continuous exposure of these workers to high concentrations of *A. fumigatus*' spores (Marsh *et al.*, 1979).

Extensive toxicological and feeding studies are required before a new SCP product can be grown on a large scale and marketed, even as fodder. These studies can take up to five years and should be undertaken by a specialist in this field (Kihlberg, 1972). Although the potential danger of spore formation during the production stage and/or the production of toxins is of prime importance in any SCP production study, any investigation of this aspect is beyond the scope of this study.

2.4.2 Aseptic growth conditions. Where pure cultures are used for SCP production, aseptic growth conditions are required to prevent contamination of the biomass with unwanted microorganisms or phage attacks (Kihlberg, 1972). Maintaining aseptic growth is expensive compared to non-aseptic growth, due to substantially higher capital and operating costs.

To overcome most of these problems, experimental processes such as the microsieve process have been developed. These processes rely on selective growth conditions and/or use selective cultivation techniques to maintain monoculture growth (Pretorius & Hensman, 1984). With these processes monocultures of filamentous microorganisms can be maintained indefinitely on sterile influents without having to maintain aseptic cultivation conditions.

2.4.3 Drying of the final product. The biomass is usually dried in steam-tube, rotary, spray, drum, tray or belt driers to between 5% and 8% moisture content (Atkinson & Mavituna, 1983). For SCP production this step must sufficiently kill desired as well as any contaminant strains. Drying, usually at temperatures above 160°C, is expensive since only a fraction of the heat can be recovered (Bailey & Ollis, 1986). Although

drying and product blending contribute significantly towards the cost of SCP production, this aspect is not dealt with here.

2.4.4 Nutritional value. When SCP was sold for human consumption during the 1970s, little had been done regarding feed and toxicological studies or marketing to convince consumers that the final product did not constitute a health hazard. Much has since been done to restore consumer confidence, and Quorn, for example, is widely accepted by the public in Britain today (Sadler, 1988). Quorn is promoted as having a high protein and fibre content but no cholesterol or animal fats.

To assess the true (market) value of the SCP produced and to compare its nutritional value with that of other, known SCPs, an amino-acid profile of the biomass is required. SCP generally lacks the nutritional value of animal protein because microbial proteins have a low methionine-amino-acid content and a high nucleic acid content (Lipinsky & Litchfield, 1974), compared to fish or meat. Methionine is a commercial product and supplementation is feasible. High nucleic acid contents in SCPs lead to poor digestibility, urinary stone formation and gout in humans (Litchfield, 1977).

Techniques can be applied to improve the nutritive and organoleptic qualities of SCP. These techniques include mechanical cell disruption, heating, (cooking and autoclaving), autolysis, plasmolysis and chemical protein extraction (Kihlberg, 1972).

Clearance to market SCP products for human consumption was refused in Italy in 1976 because of a strong consumer outcry related to

unsubstantiated fears of carcinogenic residues, hailing from the *n*-paraffin substrate, in the final product (Solomons, 1983). When using purified *n*-paraffins to produce the SCP 'Toprina' or 'Liquipron', 3,4-benzpyren, a potent carcinogen, was detected at concentrations below 1 $\mu g.l^{-1}$. This, however, is 12 to 13 times less than can be found in conventional baker's yeast.

Strict testing will be required in feeding studies, with extensive clinical and histological examinations, before a new SCP product, even if used as fodder, can be released on the market.

The most important aspects related to SCP production were discussed in the above section. Important aspects for a proper economic evaluation of producing SCP on a large scale should include mass and energy balances, drying, sterilisation, toxicological studies and determining the market value of the product. This study does not contain an economic evaluation and the above aspects will therefore not be addressed.

3. SUBSTRATE SUITABLE FOR SCP PRODUCTION

From the literature study it became evident that, in order to develop an economically viable SCP process in a free-market economy and one which can compete with agriculturally produced protein, a cheap and readily available substrate such as a waste stream containing high organic loads will be required. The following section is a summary of what is available in South Africa and what has so far been done to utilise waste effluents for SCP production.

3.1 Potential waste streams suitable for SCP production

South Africa has several "wet" industries which produce large volumes of effluent containing high organic loads. Table 1 lists three major industries.

Table 1. Major industries in South Africa which produce large volumes of organic effluent

INDUSTRY	EFFLUENT DISCHARGE		REF
	COD* ($g.l^{-1}$)	(t $COD.a^{-1}$)	
Sugar	1,5 - 3	720 000	i
Synthetic fuel	16 - 20	1 620 000	ii
Pulp and paper	0,2 - 20	13 000 000	iii

* Typical values

i Steffen *et al.*, 1990b

ii Kühn, 1989a

iii Steffen *et al.*, 1990a

A large portion of the COD of these effluents consists of easily biodegradable organic constituents, which makes them attractive for utilisation as a substrate for SCP production. Synthetic fuel effluent, for example, contains up to 13 $g.l^{-1}$ volatile fatty acids of which 70% (v/v) is acetic acid and 15% (v/v) is propionic acid (Du Preez *et al.*, 1981). Pulp and paper-mill effluents comprise between 18% and 26% (m/m) total sugars of which approximately 80% (m/m) is hexose and 20% (m/m) is pentose (Andersen, 1979).

Du Preez (1980) identified synthetic fuel (Sasol) effluent and Cloete (1990) pulp and paper-mill effluent as possible sources of substrate for large-scale SCP production in South Africa. The sugar industry also produces effluent containing high organic loads which could possibly be utilised as substrate (see Table 1).

3.2 SCP in South Africa utilising industrial wastes

No large-scale plant utilising industrial waste to produce SCP exists in South Africa, but SCP is produced on a small scale, mainly to serve as food extract or for pilot studies. The utilisation of waste as a substrate for SCP production from the following industries has been researched:

- Synthetic fuel industry. This effluent, which is produced during Fisher-Tropsch synthesis, contains mainly C_2-C_5 monocarboxylic acids and small amounts of alcohols, ketones and hydrocarbons (Kühn, 1989a).

  Du Preez (1980) was able to cultivate the bacterium *Acinetobacter calcoaceticus* as a chemostat culture from this effluent. Low biomass concentrations (5 $g.l^{-1}$ dry wt) were obtained which, in conjunction with harvesting the small-size cells, would have rendered SCP production uneconomical. To increase biomass concentration, the feed was supplemented with 20% (v/v) ethanol. When ethanol was implemented as a fuel extender for petrol in subsequent years, this supplement became too valuable and research was terminated.

  Kühn and Pretorius (1989a) were able to continuously cultivate the fungus *Geotrichum candidum* as a monoculture under non-aseptic culture conditions in a microsieve process. Almost 90% COD reductions were achieved and

biomass production rates of up to 0,57 g cells.$(l.h)^{-1}$ could be maintained in laboratory reactors operated at 28°C. A pilot reactor was built and is currently being tested. The process has the disadvantage of utilising a mesophilic microorganism which requires expensive cooling to maintain optimum operating conditions. It is also subject to low (< 10 $g.l^{-1}$) biomass concentrations because oxygen transfer becomes problematic when fungal biomass of higher concentrations is produced. Kühn and Pretorius (1989a) estimated that up to 14 000 t of SCP could be produced annually from this effluent. The use of this effluent for full-scale SCP production has reached the implementation stage.

- Pulp and paper industry. Effluents produced by this industry stem mainly from bagasse pulping and calcium sulphite pulping and contain lignins, sugars and volatile acids as the main constituents.

McKee and Quicke (1977) produced a food yeast, *Candida utilis* SATCC27D, utilising spent sulphite liquor (SSL) as substrate. The yeast grew poorly on undiluted (COD ~ 230 $g.l^{-1}$) SSL in laboratory-scale batch fermentors operated at a pH of 4,3 and a temperature of 29°C. Better results were obtained when the SSL was diluted to give a feed COD of 58 $g.l^{-1}$, and maximum yields of 0,548 g cells per gram of sugar utilised were subsequently achieved. The maximum biomass production rate that could be achieved was 0,112 g cells.$(l.h)^{-1}$. COD removals of only 1,7% were obtained and this was ascribed to the adverse composition of the SSL from the specific mill. With 1 260 $m^3.d^{-1}$ of SSL it was calculated that approximately 9 360 t of SCP could annually be produced from this effluent. McKee and Quicke (1977) concluded that high production costs would render this process uneconomical if the yeast were to be used as fodder for animals.

Kühn and Pretorius (1989b) were able to use SSL, which they diluted to obtain a feed COD of 7,8 $g.l^{-1}$, in a similar set-up as for the synthetic fuel effluent, also utilising the fungus *Geotrichum candidum*. COD reductions of up to 49% were obtained and biomass production rates of up to 0,57 g $cells.(l.h)^{-1}$ were achieved. At an optimum operating temperature of 28°C, a yield of 0,47 g cells (dry wt).g COD^{-1} removed, was achieved. From these figures it is estimated that approximately 120 000 t of SCP could be produced from this effluent annually.

- Sugar industry. Bagasse, a lignocellulosic waste, is produced by this industry. It mainly comprises cellulose (38% dry wt), hemicellulose (33% dry wt) and lignin (22% dry wt) (Walford & Purchase, 1989). The hemicellulose in bagasse is a sugar which is relatively easily hydrolysed and extracted with 0,5% sulphuric acid and atmospheric pressures at 120°C. Hemicellulose hydrolysate contains high concentrations of xylose (40 $g.l^{-1}$), acetic acid (13 $g.l^{-1}$) and glucose (2,5 $g.l^{-1}$) as major constituents (Du Preez, 1989). It is used as a substrate for SCP production.

- Holder (1987) was able to cultivate *Candida utilis* and *Geotrichum candidum* in batch processes utilising cellulose hydrolysate, which contained 40 $g.l^{-1}$ glucose. Maximum biomass production was 0,38 $g.l^{-1}$ in 12 h for *Candida utilis* and 0,4 $g.l^{-1}$ in 25 h for *Geotrichum candidum*, at an optimum operating temperature of 30°C for both organisms. Meyer *et al.* (1990) produced *Candida blankii* on a hemicellulose hydrolysate substrate as on laboratory-scale continuous culture. The yield was unacceptably low (0,14 g $cells.g\ substrate^{-1}$).

Du Preez and Kilian (1989) estimated that the bagasse from a medium-sized (250 t $cane.h^{-1}$) sugar factory would render approximately 20 000 t of SCP

per annum. If the total amount of bagasse accrued during sugar production in South Africa is utilised for SCP production, approximately 230 000 t could be produced per annum.

It is evident from the above information that South Africa possesses several organically polluted effluent streams that could be exploited for SCP production. The combined, estimated potential for SCP from the synthetic fuel, pulp and paper and sugar industries is approximately 364 000 t per annum, which would contribute significantly to decrease the estimated annual protein deficit of 400 000 t by the year 2000 (Chapter I).

From the identified effluents with potential for SCP production, SSL appears to be a good choice as substrate for SCP production. It has considerable potential for SCP production (120 000 $t.a^{-1}$) and does not require any pretreatment, such as the acid hydrolysis required for bagasse, before it can be utilised as a substrate. As there is, as yet, no full-scale SCP production plant in South Africa which utilises SSL, the potential use of this effluent for SCP production will be further investigated.

3.3 SSL as substrate for SCP production

3.3.1 Effluent quantity and quality. The pulp mill, whose effluent was used in this study produces about 5 000 $m^3.d^{-1}$ of SSL. This effluent is combined with all the washing and bleach streams produced at the plant and the final effluent then adds up to 80 000 $m^3.day^{-1}$ with an organic content of between 16 g and 26 g $COD.l^{-1}$ (Thubron, 1991). Final effluent is discarded at temperatures between 45° to 75°C and a pH of about 2,4.

Independent surveys (Oliff *et al.*, 1969) have shown that this effluent, which is discharged to sea, is harmless to the environment, although it can cause localised aesthetic nuisance conditions when residual foam, which develops during turbulent seas, is deposited on the beach.

A typical analysis of the SSL is presented in Table 2. The effluent mainly consists of sugars (hexoses and pentoses), short-chain alcohols and fatty acids, calcium lignosulphonate, sulphur dioxide and cellulose residues. Although the sugars and short-chain carbon compounds are easily biodegradable, lignosulphonates are not easily removed from SSL by microbial decomposition (Jurgensen & Patton, 1979). The reason is because lignin, which strengthens cell walls in plants, also protects the microfibrils of these walls from chemical, physical and biological attack (Goodwin & Mercer, 1986). Lignin compounds such as lignosulphonates show a similar resistance to biological degradability (Pamment *et al.*, 1979).

Table 2. Typical analysis of the calcium spent sulphite liquor used for research (Sappi/Saiccor, 1991b)

CONSTITUENT	CONTENTS AS:		CONCENTRATION ($g.l^{-1}$)
	% by wt	% of solids	
Lignin	28	53	367
Sugars (total)	7,4	14	98
Sulphur (total as S)	3,5	6,6	46
Acetic acid	0,07	0,13	0,9
Methanol	0,05	0,09	0,6
Furfural	0,03	0,04	0,3
Ethanol	<0,02	<0,05	<0,4
Sugar, sulphur and ash			
Sugars:			
Pentose - Arabinose	0,7	1,3	9
- Xylose	4,4	8,3	58
Hexose - Mannose	1,8	3,4	24
- Galactose	0,3	0,6	4
- Glucose	0,2	0,4	3
Sulphur: SO_2 (as S)	0,4	0,8	5
SO_3 (as S)	1,3	2,3	16
Ash: Total	4,2	7,9	55
CaO	3,0	5,7	39
Fe	0,01	0,02	0,1
Na_2O	0,04	0,07	0,5

3.3.2 Potential as substrate for SCP production. A rough estimate, based on experimental data by Kühn and Pretorius (1989b) (Section 3.2), of this effluent's potential as a substrate for SCP production, showed that 120 000 t of SCP can be produced annually if this effluent is used as substrate in the microsieve process.

4. SELECTIVE PROCESSES

Many reactor types and processes have been developed for SCP production, utilising substrates that range from solids to liquids and gases and employing a wide spectrum of microorganisms requiring different optimum growth conditions. Most of these processes employed a single microorganism which was grown under aseptic culture conditions (Du Preez, 1990), requiring expensive equipment.

Different microorganisms have different physiological and physical properties, which makes it possible to select, from a diverse microbial population in nature, one or more microorganism(s) that can utilise a specific effluent better than others (Pretorius, 1987). Pretorius identifies primary selection factors as physiological properties, and secondary selection factors as physical properties of the microorganism to be selected.

Physiological properties that can be varied to favour growth of a specific microorganism include environmental conditions such as pH, temperature, oxygen availability and specific nutrient concentrations, for example carbon, nitrogen and trace elements (Atlas & Bartha, 1987). Cellulose can, for example

act as a primary selection factor since it can only be utilised by organisms producing cellulase such as *Aspergillus fumigatus* and *Trichoderma reesi* (Stewart & Parry, 1981). The enrichment culture techniques (Pelzcar *et al.*, 1986) which are used by microbiologists to identify and study specific microorganisms are also based on these principles.

Secondary selection is a dynamic selection process which makes use of the physical properties of microorganisms, such as their electrical charge, mass, size and growth rate (Pretorius, 1987). A dynamic selection process has been developed at the University of Pretoria with which monocultures can be selected from a multispecies culture and maintained indefinitely under non-aseptic growth conditions (Hensman, 1985; Kühn & Pretorius, 1988b; Kühn, 1989). This process employs a cross-flow microsieve reactor to achieve dynamic selection.

The microsieve reactor. This reactor relies on a relatively large pore size (100 µm) microsieve and short hydraulic retention times (τ_h < 3 h) to selectively wash small-sized microorganisms, such as contaminating yeasts and bacteria out of the biomass whilst retaining large filamentous microorganisms such as fungi (Kühn & Pretorius, 1988b). The number of filamentous species is limited by the relatively short biomass retention times (θ_c = 1,5 to 2,5.τ_h). Because θ_c and τ_h can be separately controlled, the microsieve reactor may be considered a continuously stirred tank reactor (CSTR) with cell recycle (Pretorius & Hensman, 1984). Due to cell recirculation, higher concentrations of biomass can be maintained in this type of reactor than in an 'open' reactor, such as the CSTR without recycle. This makes the microsieve process ideally suitable for application with relatively low substrate concentrations, such as for most industrial effluents carrying organic material.

One serious drawback of the microsieve process is that oxygen becomes the limiting substrate at relatively low fungal biomass concentrations in the microsieve reactor. Wille (1992) found that it was not practical to maintain biomass concentrations exceeding 10 $g.l^{-1}$ (dry cells) with *Geotrichum candidum* because effective oxygen transfer became seriously limiting due to increased viscosity at these and higher fungal concentrations. Using Monod kinetics (Appendix B) and operational constraints of θ_c and τ_h for optimum selection, it can be shown that this process is suitable for effluents with biodegradable COD of 4 $g.l^{-1}$ or less. To prevent oxygen from becoming the limiting substrate, the substrate will have to be diluted. Dilution would normally be achieved by adding water to the feed, which means that if the substrate is a waste stream, its volume will be increased using water of a good quality and to discard the bulk afterwards. Also, water is a scarce commodity in South Africa (Department of Water Affairs, 1986) and should, if possible, not be used for such industrial applications. Alternatively the feed can be diluted with effluent from the reactor, which would require sterilisation and would therefore increase the operating costs.

The microsieve process, with its ability to maintain pure, filamentous cultures under non-aseptic culture conditions, could contribute towards the viability of a large-scale SCP process if a simple, cost-effective solution could be found to the problems associated with limited oxygen availability at high biomass concentrations.

5. CONCLUSIONS

It may be concluded from this literature study that a number of factors play a role in providing an economically viable SCP production process. Among the major

cost factors identified are substrate source, cooling, maintaining non-aseptic cultivation conditions and harvesting of the biomass.

Although various potential substrate sources have been identified, a readily available and reliable substrate source seems to exist in the form of SSL from a local pulp mill. SSL contains a large, not easily biodegradable fraction which necessitates a biodegradability study to determine its full potential as SCP.

The final effluent of the pulp mill is discharged at a temperature of approximately 50°C, which necessitates cooling if mesophilic microorganisms are to be used for SCP production. Cooling would be unnecessary if a thermophilic organism is used instead.

A cultivation method based on dynamic species selection is available so that a pure culture of a selected filamentous species can be maintained under non-aseptic growth conditions.

The filamentous nature of the biomass which is produced during the microsieve process simplifies harvesting, and less expensive equipment may be used to separate the biomass from the culture medium.

An economically viable process for SCP production could be the use of a microsieve process and employing a thermophilic filamentous fungus that can utilise SSL as the substrate. The main aims of this study was to:

- evaluate the SCP potential of SSL as substrate by determining its percentage biodegradability;

- attempt to select a thermophilic or thermotolerant filamentous fungus capable of utilising SSL as substrate;

- present a possible flow arrangement for a full-scale SCP production plant;

- define constraints for the flow arrangement that should be taken into account when designing full-scale plants.

The biodegradability of SSL and its potential as a substrate for SCP production are investigated in the following chapter.

CHAPTER III

BIODEGRADABILITY OF SPENT SULPHITE LIQUOR

1. INTRODUCTION

SSL has a complex composition of organic constituents which depend on the type of wood digested and the quantities of chemicals employed during processing (Jurgensen & Patton, 1979). For example, SSL from hardwood pulp such as wattle and *Eucalyptus* trees, contains between 20% and 25% lignin, whereas SSL from softwood pulp such as pine trees contains between 25% and 35% lignin (Swartz, 1989).

The biodegradable part of the COD is largely controlled by the sugar content, although other easily biodegradable organic constituents such as methanol, ethanol and volatile acids also contribute considerably towards the biochemical oxygen demand (BOD) (Andersen, 1979). The total sugar content in SSL varies between 18% (wt) and 26% (wt) of the total solids. Numerous researchers (Jurgensen & Patton, 1979; Lo *et al.*, 1978; Moo-Young, 1977; Kühn & Pretorius, 1989b) who used SSL as a substrate for SCP production obtained COD reductions ranging from 30% to 50%. This indicates that the total sugars could contribute more than 50% to the biodegradable COD of SSL.

Since SCP production processes are capital and energy-intensive (Litchfield, 1977), an accurate determination of the biodegradable fraction of the SSL is important to assess its potential as a substrate for SCP production.

2. BACKGROUND

Different methods and mathematical models were developed (Grady & Lim, 1980; Ekama *et al.*, 1986) to establish and describe the biodegradable fraction of the COD of domestic and industrial waste waters. These methods stipulated that influent inert soluble COD is equal to the filtered effluent of a continuously fed completely mixed reactor system operated at relatively long sludge ages of between 10 and 20 days. Methods developed by Grady and Lim (1980) and Ekama *et al.* (1986) were based on the assumption that the non-biodegradable COD of an effluent equals the soluble COD when the rate of food uptake by microorganisms is zero.

Published results (Chudoba, 1967; Grady & Williams, 1975; Baskir & Hansford, 1980; Germirli *et al.*, 1991; Sollfrank & Gujer, 1991) showed that a major fraction of soluble COD found in the effluent from biological reactors was not part of the original substrate, but due to refractory by-products generated through microbial metabolism. The biodegradable COD fraction, if based on reactor effluent COD, would analyse lower than the substrate's true biodegradability because of the COD derived from generated by-products. This would result in incorrect assessments regarding the potential of SSL as a substrate for SCP production.

To account for by-product formation, Germirli *et al.* (1991) proposed a method that determines effluent soluble COD without including the COD due to microbial metabolic products formed. By comparing the COD profiles of two batch reactors run in parallel,

one with the waste water to be tested and the other with glucose of similar COD, the initially inert fraction of the waste water can be determined. Glucose contains no initially inert fraction, so that the minimum COD measured in the reactor fed with glucose will therefore be due to microbial metabolic products that are formed.

Two methods, the one proposed by Grady and Lim (1980) and the other by Germirli *et al.* (1991), were used in this study to determine the soluble COD of SSL. An indication could be obtained of the by-products produced by the microorganism, because the first method does not account for refractory by-products formed whereas the second does. It is important to establish the true biodegradability of the SSL in order to assess its potential for SCP production.

3. MATERIALS AND METHODS

3.1 Initial preparation

3.1.1 Inoculum. To include the widest possible diversity of suitable microorganism species, samples for the preparation of an inoculum were collected from garden topsoil, garden compost heaps and various sewage samples. Approximately 1 l 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 2 mm-opening size screen and 2 l each of raw domestic sewage and mixed liquor from the aeration basin of an activated sludge plant were added. This gave approximately 5 l of a mixed species suspension which formed the inoculum.

3.1.2 Feed. SSL as a subtrate was diluted with tap water to obtain a total COD of approximately 7 $g.l^{-1}$ and supplemented with nutrients as described by Tabak and Cooke (1968) to give a COD:N:P ratio of 100:15:3 (m/m). This composition represented the approximate stoichiometric requirements for the conversion of carbohydrates into microbial cells. After the addition of nutrients, the pH was adjusted to 5,5 with diluted H_2SO_4 because this is the pH at which most microorganisms can survive and grow (Atlas, 1980). This substrate is designated diluted spent sulphite liquor (DSSL).

3.1.3 Adapting microbial population to DSSL. The 5 l inoculum and 15 l of substrate were filled into a batch reactor through which air was bubbled at a superficial rate of 300 $m.h^{-1}$ (Germirli *et al.*, 1991) and where the temperature was maintained at 30 ± 1°C. A third of the volume of the reactor contents was replaced daily with fresh feed for the next 16 days, to obtain a heterogeneous microbial population. The DSSL-adapted culture was then adapted, as before, for 21 days to a substrate containing 50% (v/v) DSSL and 50% (v/v) glucose of a similar concentration as the waste water.

3.2 Experimental

3.2.1 Reactors. Two similar, 20 l aerated batch reactors were run in parallel, one with DSSL and the other with glucose as the substrate. The initial substrate COD concentration in both reactors was 7 $g.l^{-1}$. The temperature in the reactors was maintained at 30°C and the initial pH was 5,5. Both reactors were inoculated with 0,5 $g.l^{-1}$ (dry wt) of DSSL/glucose-adapted biomass.

3.2.2 Sampling. 250 ml samples were taken at 2 h intervals for the 72 h duration of the experiment. Distilled water was used to compensate for evaporation losses.

3.2.3 Analytical methods. The samples were filtered and analysed in duplicate for COD and biomass (SS) according to *Standard Methods* (1985).

3.2.4 Data processing.

Grady and Lim method. Grady and Lim (1980) showed that the substrate removal rate, q, for a batch reactor can be expressed with reasonable accuracy by the following equation:

$$q = \frac{(S_{to} - S_t)}{t.X_t} \quad \ldots\ldots (5)$$

where S_{to} and S_t represent biodegradable COD at the start and at time t respectively and X_t represents the cell concentration at time t. If q is plotted as a function of reactor soluble COD, the curve will have a positive abscissa intercept if a portion of the COD is non-biodegradable, equal in magnitude to the value at the intercept. Thus, this method assumes that the non-biodegradable COD is at the point where the specific rate of substrate removal by the microorganisms is zero.

Germirli *et al.* method. The profile of the reactor soluble COD as it changes with time (abscissa) is plotted for both reactors, the one with glucose as the substrate and the other with waste water. The first point of minimum COD is obtained from the graph for both systems. The biodegradable fraction is:

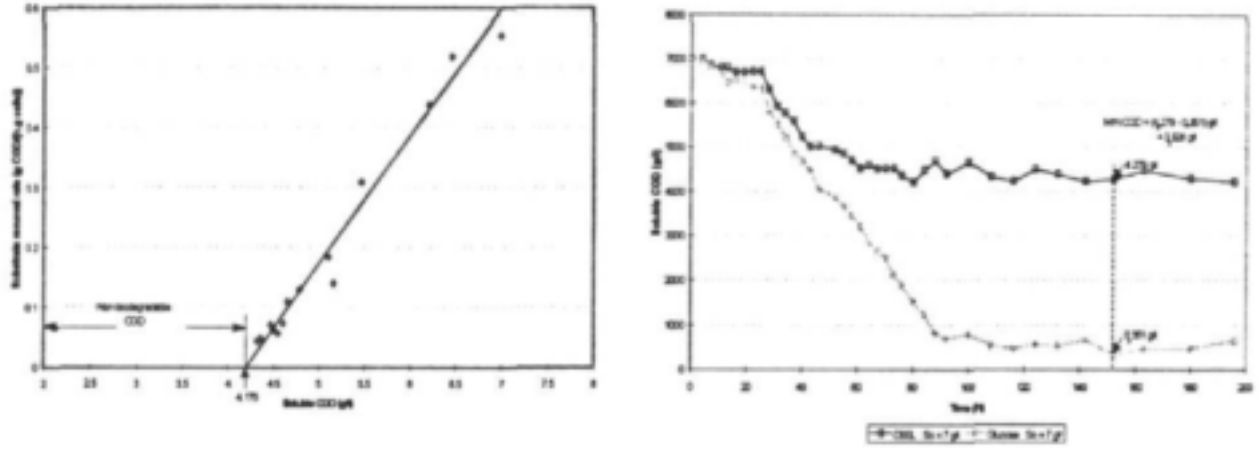
$$\frac{\text{initial COD} - (\text{COD waste water} - \text{COD glucose})_{min}}{\text{initial COD}} \quad \ldots\ldots (6)$$

The above analytical methods were applied to the same data set to compare the biodegradability figures for the SSL.

3.2.5 Statistical methods. Quattro Pro (Ver. 5) was used for the linear regression analysis.

4. RESULTS

The data obtained during the biodegradability studies by means of two different analytical methods were processed and are graphically depicted in Figure 1.



a) Grady and Lim method

b) Germirli *et al.* method

Figure 1. Determining minimum substrate soluble COD

Figure 1a) illustrates the substrate removal rate *vs* reactor soluble COD to determine the biologically inert fraction of SSL in accordance with the Grady and Lim method for an initial reactor soluble COD of 6,983 $g.l^{-1}$. Figure 1b) shows the COD *vs* time profile for two reactors, both with an initial reactor soluble COD of 6,983 $g.l^{-1}$, but fed with SSL and glucose respectively, in accordance with the Germirli *et al.* method. The results are summarised in Table 3.

Table 3. Biodegradability of DSSL with different analytical methods

METHOD	INITIAL COD ($g.l^{-1}$)	MINIMUM COD ($g.l^{-1}$)	COD REMOVAL (%)
Grady and Lim, 1980	6,983	4,175	40,2
Germirli *et al.*, 1991	6,983	3,928	43,7

5. DISCUSSION

The biodegradable COD obtained by the Germirli *et al.* method was found to be 43,7% which is slightly higher than the 40,2% (Table 3) obtained by means of the Grady and Lim method. A possible explanation could be that the latter method does not account for refractory by-products formed by the microbial culture. The difference (3,5%) is small and, for practical purposes, neglegible. The reason why this difference is small compared to observations by others (Eckenfelder, 1980; Ekama *et al.*, 1984; Germirli *et al.*, 1991) could be due to the longer sludge ages employed by these researchers.

Their research was based on sludge ages similar to those maintained in activated sludge plants for the treatment of municipal waste water, generally between 10 and 20 days (Eckenfelder, 1980), whereas the above experiments are terminated when a first minimum COD is observed, typically within the first 3 days.

With the Germirli *et al.* method the profile of COD removal against time obtained for both substrates, glucose and waste water, is very similar for the first approximately 40 hours (Figure 1b). This is to be expected because the SSL contains a high percentage of fermentable sugars (Table 2) and these sugars will be broken down in a similar way as glucose (Andersen, 1979).

The 40% to 44% biodegradability of the SSL obtained during these experiments falls well within the range of 30% to 50% found by others (Section 1). However, Kühn and Pretorius (1989b) were able to obtain COD removals of up to 49% with SSL from the same mill and an initial COD of 7,8 $g.l^{-1}$ when they used the microsieve process with a monoculture of *Geotrichum candidum*. With a heterogeneous culture as that used in the above experiments, one would expect even higher substrate removals. A possible explanation could be that the organic composition of the SSL used by Kühn and Pretorius differed significantly with regard to easily biodegradable substances from the one used for these experiments. The concentration of easily biodegradable constituents in the SSL could fluctuate due to different batches of timber used as raw material and with seasonal changes (Jurgensen & Patton, 1979).

For all theoretical calculations the biodegradable fraction is assumed to be 40% for the pulp-mill effluent. The potentially available substrate COD of the final effluent stream (80 000 $m^3.d^{-1}$; average COD ~ 20 $g.l^{-1}$) of the pulp mill whose SSL was used in this study, would then be approximately 233 600 t of biodegradable COD per annum.

6. CONCLUSION

The two analytical methods applied to determine the biodegradable fraction of SSL gave similar values, namely 40,2% (Grady and Lim method) and 43,7% (Germirli *et al.* method). The reason why this difference was not larger, considering the different approaches to refractory by-products formed by the two methods, was ascribed to the relatively short biomass retention times employed during the experiments.

When only the total COD of the SSL is known, its biodegradable fraction is assumed to be 40%. This figure will be used for reactor modelling to estimate the value of SSL as substrate for large-scale SCP production and any theoretical calculations where biodegradable COD of the pulp-mill's effluent is employed.

The selective cultivation of fungi growing at higher than the ambient temperatures is discussed in the following chapter.

CHAPTER IV

SELECTION OF A FUNGUS CAPABLE OF GROWING AT ELEVATED TEMPERATURES

1. INTRODUCTION

A variety of microbial species can utilise the organic compounds contained in SSL. An abundance of easily fermentable wood sugars (Table 2) favours yeast and bacterial growth (Gold *et al.*, 1981). A filamentous microorganism that would display optimal growth at temperatures between 45° and 75°C (Chapter II) was sought for use in the microsieve process. The added ability to grow at elevated temperatures, besides reducing cooling costs, would exert an additional, primary selection pressure on the system (Pretorius, 1987) that could promote monoculture growth. Fewer microbial species can for example grow at thermophilic than at mesophilic temperatures (Atlas, 1980).

1.1 Possible microorganisms

The mentioned temperature range of 45° to 75°C typically falls within thermophilic organisms' optimal growth temperatures (Atlas, 1980) and a suitable microorganism for the envisaged application could therefore be expected to be a thermophile. Thermophilic microorganisms often only grow at temperatures above 40°C with an upper growth temperature of about 99°C.

Although many thermophiles can survive low temperatures (-60°C) and are routinely found in frozen Antarctic soils, or such high temperatures (250°C) as occur in ocean thermal vents, optimal growth temperatures are 55°C to 60°C.

In nature, microorganisms living at high temperatures were isolated from marine geothermal sites, geysers, volcanoes, boiling outflows of geothermal power plants and in hot springs (Borman, 1991). Most of these are bacteria of the genera *Thermus*, *Sulfolobus* and *Bacillus stearothermophilus* (Atlas, 1980). Only a few fungal species of *Humicola, Malbranchea, Mucor, Talaromyces* and *Torula* can be classified as true thermophiles (Hudson, 1986). Several other species can live and survive at temperatures above 45°C, for example *Aspergillus fumigatus*, but are not strictly thermophilic organisms and are therefore classified as thermotolerant (Domsch *et al.*, 1980).

The use of thermotolerant microorganisms for SCP production has been investigated. A batch process proposed by Gregory *et al.* (1976) and developed by Santos *et al.* (1983) was used to grow *Aspergillus* sp. on rasped cassava roots at 45°C in 84% humidity. Several researchers (Coutts & Smith, 1976; Jain *et al.*, 1979) experimented with thermophilic organisms such as *Sporotrichum thermophile, Humicola insulens, Malbranchea pulchella, Mucor miehei* and *Torula thermophila* in microcultures (batch) to degrade cellulose and lignin. However, no reference could be found to a continuous, suspended-growth culture of filamentous microorganisms at temperatures of 40°C and above.

1.2 Suitability of an organism for large-scale SCP production

The ideal organism for large-scale SCP production should have some specific growth and cellular properties such as:

- The ability to utilise a variety of organic compounds as substrate, preferably most organics contained in industrial effluents (Litchfield, 1977);

- It should be easily adaptable, with little or no adjustment, to the prevailing physical and chemical conditions of the available substrate (Kihlberg, 1972). The most important conditions being temperature, pH and viscosity;

- Simple, basic nutritional requirements that do not necessitate the addition of expensive growth stimulants (Solomons, 1983);

- A high cell yield coefficient and a low cell maintenance coefficient that will minimise microbial heat generation (Abbott & Clamen, 1973);

- Easy to harvest (Bailey and Ollis, 1986);

- A cellular composition that is of nutritional value to augment animal as well as human diets (Lipinsky and Litchfield, 1974);

- No serious safety or health hazard during production, for example pathogenic strains or carcinogenic and toxic by-products (Kihlberg, 1972);

The purpose of this study was to search for an organism that will grow on SSL and possess all (or most) of the listed properties for large-scale production of SCP.

2. MATERIALS AND METHODS

2.1 Selection of monocultures

2.1.1 Experimental set-up. A low-pressure airlift fermentor with external loop (draft tube) and a 100 µm tubular stainless steel microscreen positioned inside the external tube was used as experimental reactor (Figure 2) for selecting easily harvestable (filamentous) microorganisms.

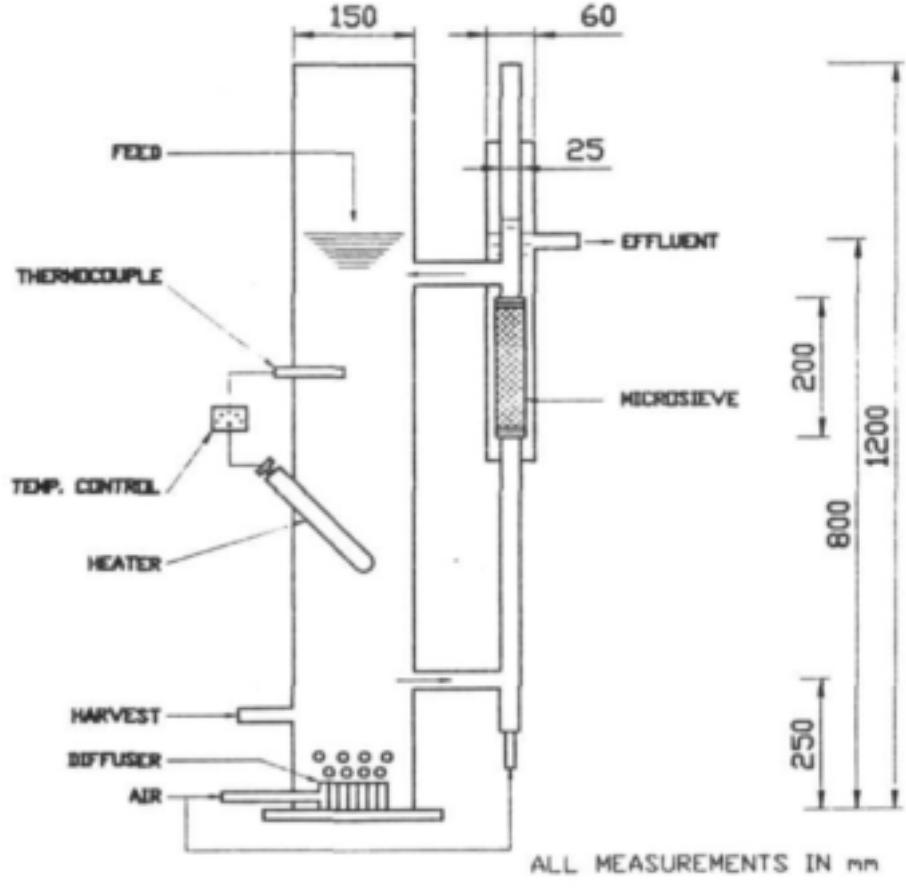


Figure 2. Experimental cross-flow microsieve reactor

This reactor was similar to the cross-flow microscreen reactor described by Kühn and Pretorius (1989a) except that no internal draft tube was provided to minimise the possible attachment area for unwanted microorganisms. The temperature in the reactor could be set at any value between room temperature and 70°C and was automatically controlled to within ±0,2°C. (Thermostat: Brainchild Model BTC-1010.) The reactor was fitted with the necessary feed supply and biomass harvesting pumps. Air was obtained from the main laboratory compressed-air supply.

2.1.2 Initial inoculum and enrichment. An inoculum was prepared as before (Chapter III). The reactor was filled with approximately 5 l of the inoculum and topped up with DSSL to its working capacity. The pH was adjusted to 5,5 as before (Chapter III), because most fungi can grow at this pH (Atlas, 1980; Domsch *et al.*, 1980). During species selection the pH remained constant within ± 0,25 units and was therefore not automatically controlled. Air at a superficial rate of ca 300 $m.h^{-1}$ was bubbled through the reactor.

Unwanted bacteria were suppressed by adding three capsules (125 mg each) of chloramphenicol during start-up. The start-up temperature was 30°C and it was stepwise increased to 45°C, with a 5°C temperature rise introduced every eight hours. This was done to allow the spores of possible thermophilic organisms to germinate and grow.

2.1.3 Dynamic selection. After 12 h of static enrichment at 45°C, feed and biomass harvesting commenced according to the operating schedule shown in Table 4. The substrate concentration was increased as indicated in Table 4 so that the microorganisms able to tolerate higher concentrations of lignin compounds would be selected.

Table 4. Feed and biomass harvesting during dynamic fungus selection

TIME AFTER DYNAMIC START-UP (h)	BIODEGRADABLE FEED COD ($mg.l^{-1}$)	HYDRAULIC RETENTION TIME (h)	BIOMASS RETENTION TIME (h)
0 - 12	0	∞	∞
12 - 36	800	32	∞
36 - 66	2 500	24	∞
66 - 90	2 500	18	24
90 - 114	4 000	9	24
114 - 138	4 000	6	18
>138	4 000	3	varying

2.2 Monitoring steady-state system changes

At least three biomass retention times were allowed for the system to reach steady-state conditions before the first set of samples was taken. After allowing at least another cell retention time to lapse, the second set of samples was taken. Both sets were then analysed for the parameters required, for example COD and suspended solids (SS). While the results were awaited, no changes were made to the operating parameters for the experimental reactor. The analytical results were accepted if found to differ by less than ±1%, otherwise they were rejected and the sampling procedure was repeated. Operating parameters for the reactor were only changed when satisfactory consecutive analytical results were prevalent.

2.3 Temperature and pH variations

The effect of pH and temperature on the growth of biomass, measured in terms of productivity (g cells.$(l.h)^{-1}$), was determined. General operating conditions for the reactor were fixed: Substrate COD concentration at 10 $g.l^{-1}$; τ_h at 3 h and θ_c at 9 h.

Once steady-state growth was obtained (Section 2.2), the operating parameters were stepwise changed as shown in Table 5.

Table 5. Stepwise changes in operating parameters

PARAMETER VARIED	RANGE		STEP	FIXED PARAME= TER AND VALUE
	MIN	MAX		
Temperature (°C)	40	50	2,5	pH = 5,0
pH	4,0	7,5	0,5	Temp = 45°C

The temperature and pH were maintained at particular set points with a thermostat and a pH-stat (E & H conduct model, Stuttgart) respectively. To lower the pH, 5 N H_2SO_4 was used, and to increase the pH, 5 N NaOH was used.

2.4 Analytical methods

2.4.1 Flow rates. The feed and effluent flow rates were calculated from measurements of the volume of liquid collected (in a measuring cylinder) for a specific period.

2.4.2 Chemical analysis. The COD of the inflow, effluent and the total SS inside the reactor was determined (*Standard Methods*, 1985).

2.4.3 Protein content. Washed, freeze-dried biomass was used for the analysis. Crude protein was determined in duplicate by the micro-Kjeldahl method (Horwitz, 1975) and the amino-acid profile obtained on acid-digested samples was analysed with a Beckman 121 M amino analyser.

2.4.4 Microscopic observations. These observations were made on live cultures taken from the reactor using phase contrast illumination.

2.4.5 Identifying dominating species. Biomass was taken from the reactor contents, streaked on Rose Bengal (Difco) agar plates and incubated at 45°C. Pure cultures of the dominating species were isolated and identified according to Onions (1982).

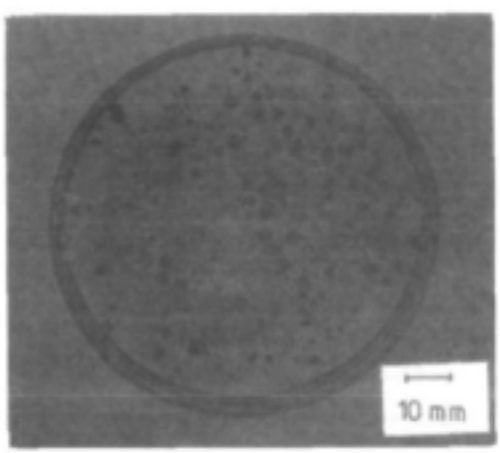
3. RESULTS AND OBSERVATIONS

No visible increase in bacterial numbers was observed in the reactor during static enrichment. This was probably due to the inhibitory effect of

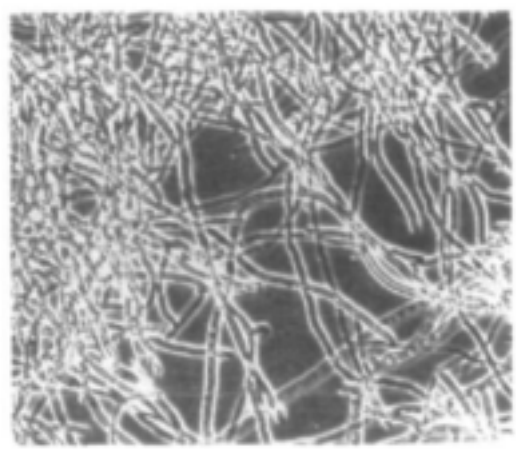
chloramphenicol which suppresses the development of fast-growing bacteria. During dynamic selection bacteria and yeasts were removed from the reactor biomass due to the selective pressure placed on the system by the combination of hydraulic residence time and microsieve size (Pretorius & Hensman, 1984). The growth of fungal hyphae could be observed in the reactor after 12 h of static enrichment at 45°C.

3.1 Selection and identification

Once feeding commenced, smooth compact 'sclerotic'-type pellets as described by Metz and Kossen (1977), varying from 1 mm to 5 mm in diameter (Figure 3a), dominated the biomass. This type of fungal growth dominated the biomass until biomass harvesting started about 66 h after feeding commenced, when the smooth, well-developed pellets changed to a pulp-like fungal mass (Figure 3b). The fungal mass dominated during further variations in τ_h and θ_c. The dominating fungus was identified as *Aspergillus fumigatus* (Onions, 1982).



a) Sclerotic pellets

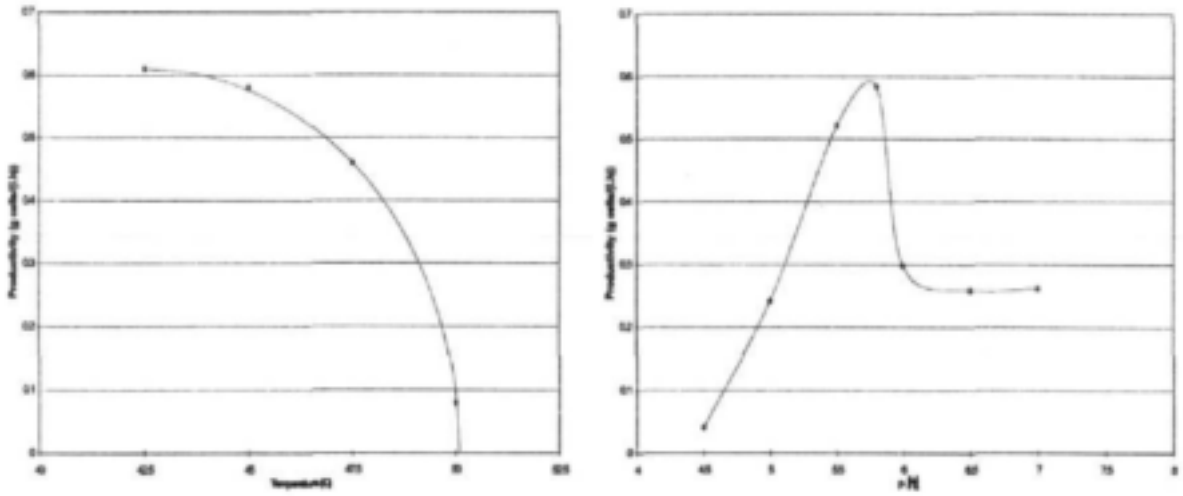


b) Pulp-like fungal biomass

Figure 3: Biomass during selection of thermophilic fungi

3.2 Temperature and pH

Figure 4 (a and b) shows the effect of temperature and pH on the productivity (biomass production rate) of the identified *Aspergillus* sp.

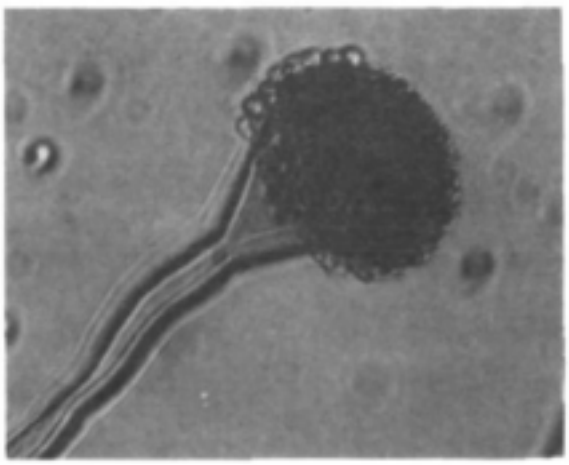


a) Effect of temperature b) Effect of pH

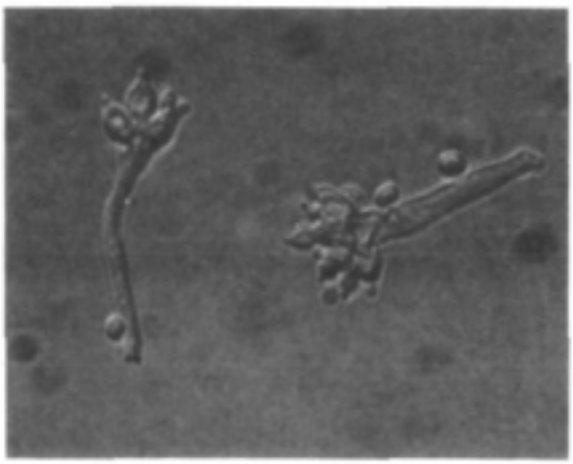
Figure 4. Effect of temperature and pH on biomass production

3.2.1 Effect of temperature. Whereas essentially a monoculture of *A. fumigatus* was microscopically observed and verified on streaked-plate agar cultures at temperatures of 45°C and above (Figure 5a), more than one filamentous fungus was observed at temperatures below 43°C.

At 40°C conidial structures which differ significantly from normally observed *A. fumigatus* could be observed on streaked-plate cultures (Figure 5b). No conidial heads or spore formation was ever observed in the biomass of the continuous culture reactors under submerged conditions. Spore formation as shown in Figure 5 occurred when the biomass was streaked on agar plates for identification purposes.



a) *Aspergillus fumigatus*



b) Unknown *Aspergillus* sp.

Figure 5. Streaked-plate cultures

3.2.2 Effect of pH. At pH values between 5 and 5,8 microscopically observed mycelia appeared "healthy" as described by Metz and Kossen (1977), with little or no vacuoli. Outside this pH range the mycelia appeared thicker, more granular and with an abundance of vacuoli. At pH values above 6,5 bacterial contamination became excessive.

3.3 Crude protein content and amino-acid profile

The results of the micro-Kjeldahl and amino-acid analyses for *A. fumigatus* are shown in Table 7. To compare the amino-acid profile of *A. fumigatus* with that of other protein sources, similar analyses results for *Geotrichum candidum* (by Kühn and Pretorius, 1989b) and for *Candida utilis*, soybean meal and fishmeal (by Lategan *et al.*, 1980) were also included.

Table 7. Comparison of amino-acid profile of *A. fumigatus* with that of different protein sources

AMINO ACID (g.(100 g)$^{-1}$)	*A. fumi= gatus*	*G. can= didum* *	*C. utilis***	SOYBEAN MEAL**	FISH MEAL**
Alanine	2,8	4,7	4,1	2,1	4,2
Argenine	2,8	6,2	3,5	3,5	3,7
Aspartic acid	3,6	2,2	7,0	5,4	6,2
Cystine	ND	0,3	0,6	0,6	0,7
Glutamic acid	5,4	5,0	8,5	8,7	8,9
Glycine	2,1	4,0	3,2	2,1	3,9
Histidine	0,9	0,6	1,5	1,4	1,5
Isoleucine	1,9	2,1	3,7	2,1	3,2
Leucine	3,1	3,1	5,5	3,6	5,0
Lysine	2,6	4,9	5,5	2,9	4,9
Methionine	0,7	0,2	1,1	0,6	1,9
Phenylalanine	1,7	1,5	3,4	2,4	2,9
Proline	2,1	1,8	2,6	2,2	2,9
Serine	1,9	2,6	3,6	2,6	3,0
Threonine	1,6	2,5	3,8	2,5	3,0
Tyrosine	1,2	1,0	2,8	1,9	2,3
Valine	2,2	2,7	4,1	2,1	3,7
% N	7,9	8,8			
% Crude protein+	49,4	55,0			
% True protein	>36,5	45,6	65,0	47,0	66,0

ND - not determined

\+ Kjeldahl x 6,25

* Kühn and Pretorius, 1989b

** Lategan *et al.*,1980

4. DISCUSSION

It was possible to select and cultivate a fungus that is capable of growing at 45°C in a continuous process. The dominating fungus was identified (according to Onions, 1982) as *Aspergillus fumigatus*. This fungus is not a true thermophile but rather a thermotolerant fungus, since it grows well at 20°C and can survive pasteurisation at 63°C for 25 minutes (Domsch *et al.*, 1980).

4.1 Temperature dependence

At temperatures below 40°C the biomass consisted of a variety of microbial species and *Geotrichum* sp. and *Aspergillus* sp. could be identified as dominating fungi in the mesophilic temperature range. At temperatures between 40°C and 45°C *Aspergillus* sp. showed optimum growth and productivity (Figure 4a). Above 45°C productivity dropped sharply and no sustainable growth of this fungus was possible at temperatures above 50°C. However, the fungus was able to tolerate a temperature of 55°C for more than four hours. The ability of *Aspergillus* sp. to tolerate temperatures above 50°C and below 40°C for a prolonged period makes this fungus, for application in large-scale processes, less sensitive to operational problems than strictly mesophilic or thermophilic microorganisms would be.

4.2 pH dependence

The optimum pH for the cultivation of *A. fumigatus* was between 5,25 and 5,75 (Figure 4b). *A. fumigatus* grows poorly at a pH below 5,25 and suppression of bacterial growth with a low pH as selection aid was not as significant as

observed by Kühn and Pretorius (1989b) with the selective cultivation of *Geotrichum* sp. on an effluent containing volatile fatty acids.

4.3 Health hazard

Although no conidiospore formation was observed in the liquid cultures, SCP production utilising *A. fumigatus* should be further investigated for its toxicological and pathogenic properties. Fungi capable of growth at body temperature could be a biohazard for unusually susceptible individuals (Chick *et al.*, 1975). *Aspergillus*' spores cause the disease group known as aspergillosis (Alexopoulus & Mims, 1979). Marsch *et al.* (1979) stated, however, that *A. fumigatus* is an opportunistic pathogen that grows and causes aspergillosis only rarely if ever in individuals not already suffering from some other respiratory difficulty.

4.4 Value as protein

The true protein content of more than 36,5% for *Aspergillus* sp. was lower than the 45,5% observed by Kühn and Pretorius (1989b) for *Geotrichum candidum* (Table 7). 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 render the SCP of *Aspergillus* sp. also superior to SCP from *G. candidum* (Nell, 1991).

5. CONCLUSION

It was possible to select a thermotolerant fungus, *A. fumigatus*, which was able to grow continuously at 45°C as a submerged culture utilising DSSL as the substrate. This thermotolerant fungus could be of commercial advantage because of a reduced need for cooling and additional selection pressure exerted by the elevated temperature which limits the number of microbial species present in the biomass.

A. fumigatus is considered an opportunistic pathogen (Marsch *et al.*, 1979) and although no conidiospore formation was observed in the submerged cultures, SCP produced from *A. fumigatus* should be further investigated for its toxicological and pathogenic properties.

The relatively good amino-acid profile of this fungus, particularly the slightly higher concentrations of lysine and methionine compared to concentrations in *Geotrichum candidum* (Nell, 1991), makes *A. fumigatus* valuable as a protein source to supplement animal feeds.

The thermotolerant fungus *A. fumigatus* should be seriously considered for commercial production of SCP under continuous, non-aseptic culture conditions.

CHAPTER V

BIOMASS PRODUCTION OF *ASPERGILLUS FUMIGATUS* ON SPENT SULPHITE LIQUOR IN NON-ASEPTIC CULTURE CONDITIONS

1. INTRODUCTION

A. fumigatus was selected and cultivated as a monoculture in non-aseptic culture conditions on highly diluted SSL, utilising the microsieve process. This thermotolerant fungus, if continuously grown as a monoculture for SCP production, has distinct advantages compared to fungi that only grow in the mesophilic temperature range. With optimum growth at 45°C, continuous cultivation of the fungus could be more economical than with mesophilic fungi. Less biologically generated heat has to be removed and cooling of the SSL's relatively high discharge temperature is not required.

Various reactor and process configurations are possible to grow microorganisms on a large scale. In this chapter two reactor configurations are compared in terms of their suitability for the continuous cultivation of *A. fumigatus* on SSL.

2. THEORETICAL BACKGROUND

2.1 Flow configurations

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

2.1.1 CSTR with cell recycle. In this reactor θ_c and τ_h are separately controlled and, to prevent losing the biological culture, the reactor is always operated with $\theta_c > \tau_h$. The result is that the biomass concentration (X) is also higher than S_o. By employing the microsieve reactor, which could be compared to a CSTR with cell recycle, it was possible to select and continuously cultivate *A. fumigatus* as a monoculture in non-aseptic culture conditions.

Limited oxygen transfer, already observed at relatively low suspended growth concentrations in a CSTR with recycle, renders the basic microsieve reactor unsuitable for operation at high feed concentrations (Wille, 1992). Although this could be prevented by diluting the feed, such a step would be detrimental to the economics of the process. To overcome this deficiency, a CSTR without cell recycle could be employed.

2.1.2 CSTR without cell recycle. Since θ_c and τ_h are the same in this reactor and the yield (mass of cells produced per mass of COD utilised, Y_g) is generally less than unity, X will always be lower than S_o.

may be used to predict the theoretical biomass production in any reactor configuration (Grady & Lim, 1980). Mathematical models may also be used for upscaling to full-scale reactors and for preliminary estimates of biomass production in full-scale plants. *A. fumigatus'* growth kinetic parameters were determined and steady-state equations were compiled to predict the theoretical reactor behaviour for the different flow configurations. To test the validity of using theoretically predicted values for X, S and r_O, these values were also determined experimentally.

2.3 Mathematic modelling

The mathematical modelling of CSTRs and CSTRs in series configurations is fully covered by Grady and Lim (1980). Table 8 gives the applicable equations for the different CSTRs used in this study.

Unfortunately the selective pressure (Pretorius, 1987) exerted through the microsieve by washing out smaller, contaminating microorganisms, is lost when employing such a reactor.

Because the selective pressure of the microsieve is lost in the latter configuration, it does not necessarily mean that this CSTR cannot be employed for monoculture growth in non-aseptic conditions. The higher temperature at which *A. fumigatus* is cultivated serves as a primary selection factor (Pretorius, 1987) and may suffice to maintain monoculture growth in a CSTR without recycle. The efficiency of a temperature of 45°C as a selection factor in a CSTR without cell recycle was further investigated.

Another option to reduce the biomass concentration or the amount of cellular mass would be to combine the microsieve reactor with a CSTR without cell recycle in a two-stage flow configuration. In such a series reactor set-up the benefit of the selection pressure of the microsieve process could be retained with minimal dilution: The first reactor, a CSTR with cell recycle, serves as selector to maintain a monoculture whereas the second reactor, a CSTR without recycle, is used to produce biomass (SCP). The monoculture harvested from the first (selector) reactor is continuously reintroduced, as an inoculum, into the second (producer) reactor. The latter runs at lower biomass concentrations resulting in better oxygen transfer. The behaviour of an experimental selector/producer reactor set-up was studied.

2.2 Growth kinetic parameters

If the growth kinetic parameters of a microorganism are known, mathematical models expressing microbial growth such as those developed by Monod (1949) may be used to predict the theoretical biomass production in any reactor

Table 8. Applicable equations for different reactors (Grady and Lim, 1980)

PARAMETER	SINGLE CSTR	CSTR WITH CELL RECYCLE	PRODUCTION REACTOR
Specific growth rate, μ	$\frac{1}{\tau_h} + b$	$\frac{1}{\theta_c} + b$	$\frac{1}{\tau_2} + \frac{X_1}{\tau_{12} X_2} + b$
Substrate concentration, S	$\frac{\mu . K_s}{\mu_m - \mu}$	$\frac{\mu . K_s}{\mu_m - \mu}$	$\frac{-B \pm \sqrt{(B^2 - 4.A.C)}}{2.A}$
Total biomass concentration, X	$\frac{Y_g . (S_o - S)}{\mu . \tau_h}$	$\frac{Y_g . (S_o - S)}{\mu . \tau_h}$	$\frac{\frac{X_1}{\tau_{12}} + Y_g(\frac{S_1}{\tau_{12}} + \frac{S_o}{\tau_{02}} - \frac{S_2}{\tau_2})}{\frac{1}{\tau_2} + b}$
Oxygen utilisation rate, r_o	$\frac{(S_o - S)(1 + b.\tau_h - ß.Y_g)}{\tau_h (1 + b.\tau_h)}$	$\frac{(S_o - S)(1 + b.\theta_c - ß.Y_g)}{\tau_h (1 + b.\theta_c)}$	$\frac{S_1}{\tau_{12}} + \frac{S_o}{\tau_{o2}} - \frac{S_2}{\tau_2} + \frac{ß_1 . X_1}{\tau_{12}} + \frac{ß_2 . X_2}{\tau_2}$

where:

$$A = \mu_m \left(\frac{1}{\tau_2} - b\right)$$

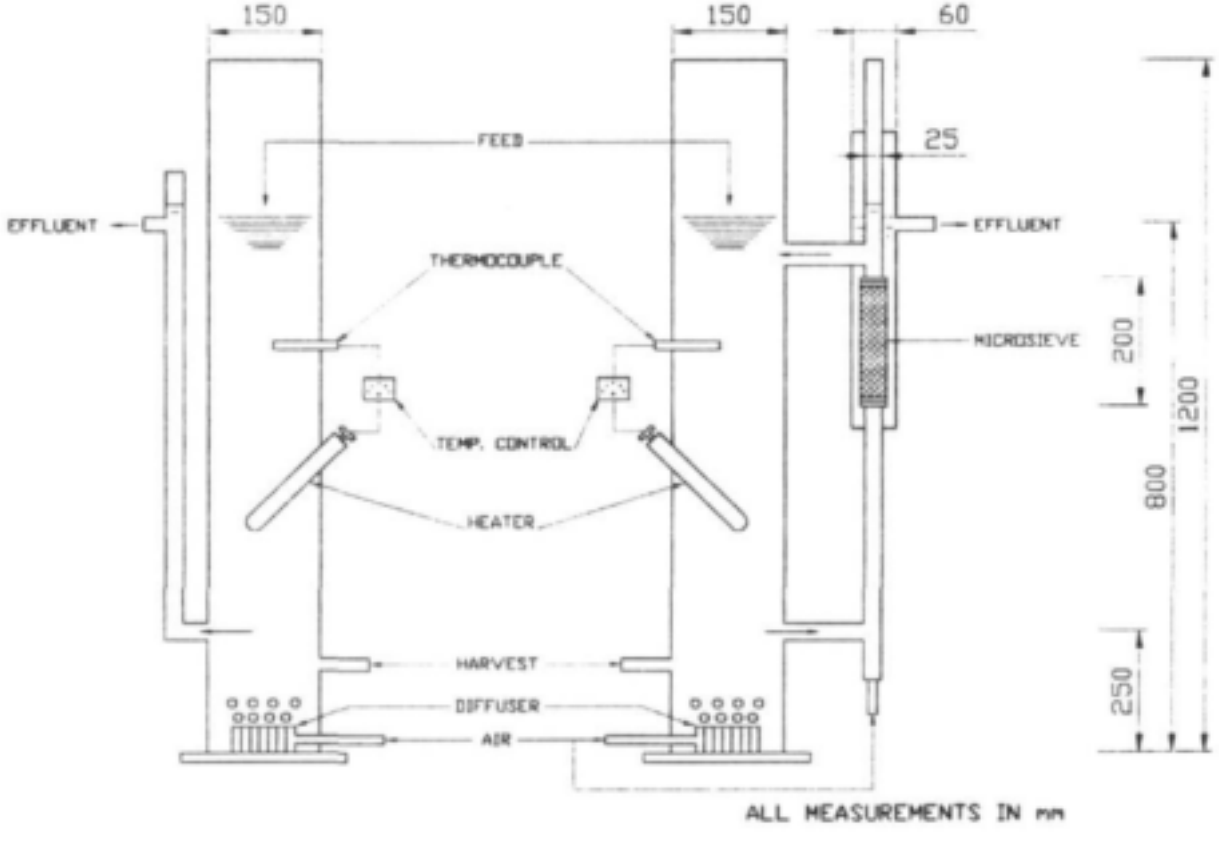
$$B = -\frac{\mu_m . X_1 . \tau_2}{Y_g . \tau_{12}} - \left(\frac{1}{\tau_2} + b\right) . K_s - \left(\mu_m - \frac{1}{\tau_2} - b\right) \left(\frac{S_1 . \tau_2}{\tau_{12}} + \frac{S_o . \tau_2}{\tau_{02}}\right)$$

$$C = K_s . \left(\frac{1}{\tau_2} + b\right) \left(\frac{S_1 . \tau_2}{\tau_{12}} + \frac{S_o . \tau_2}{\tau_{02}}\right)$$

3. MATERIALS AND METHODS

3.1 Bioreactor types

Two identical experimental reactors were used, one with an unrestricted outflow (CSTR without cell recycle - Figure 6a) and one equipped with a cross-flow microsieve on the outlet (CSTR with cell recycle - Figure 6b).



a) CSTR without cell recycle b) CSTR with cell recycle

Figure 6. Experimental reactors

Each reactor was fitted with its own variable rate feed supply pump, air supply and temperature controllers. Biomass harvesting on the microsieve bioreactor was carried over with a variable speed pump. Process equipment was selected and sized to be employed as a scaled-down version of a full-scale plant.

3.2 Flow arrangements

The performance of the reactors in three different configurations was evaluated, namely a CSTR without cell recycle, a CSTR with cell recycle, and a chain in series of a CSTR with cell recycle followed by a CSTR without cell recycle. The latter arrangement was obtained by combining the previous two reactors in an experimental set-up as shown in Figure 7.

The set-up shown in Figure 7 will be called "CSTRs in Series" to simplify the terminology. The CSTR with cell recycle is the 'Selector' reactor, whereas the CSTR without cell recycle is the 'Producer' reactor. This terminology corresponds with the reactor function described in Section 2.1 of this chapter.

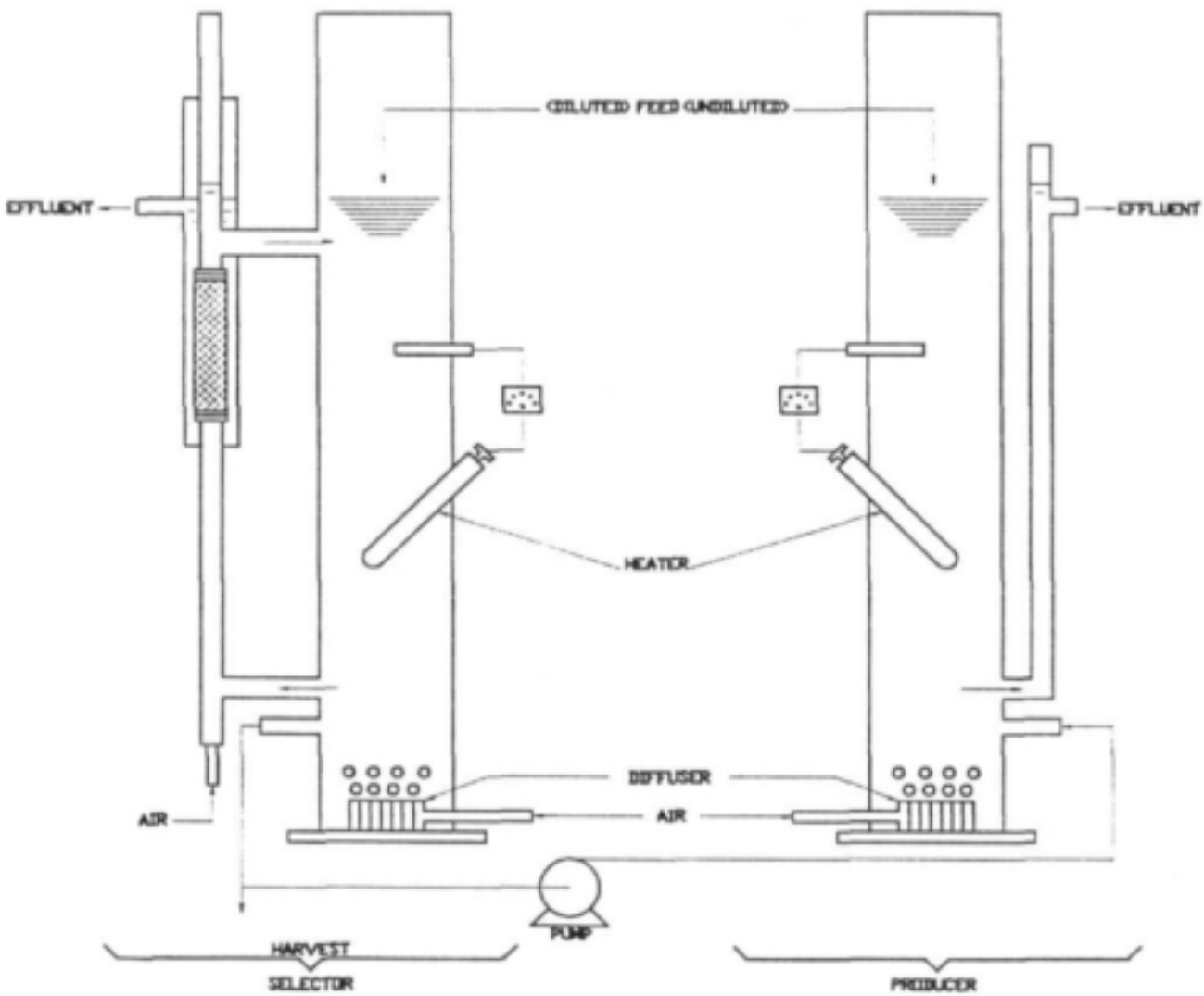


Figure 7. Experimental CSTRs in series (Selector/Producer)

3.3 Feed

DSSL, supplemented with nutrients as before, was used as the feed. The pH was adjusted to fall within the optimum range for cultivation of *A. fumigatus*, namely 5,5.

To ensure that the biodegradable matter (and not oxygen) would remain the growth limiting substrate during continuous biomass cultivation, feed to the Selector was diluted to approximately 7,5 $g.l^{-1}$ total COD (ca. 3 $g.l^{-1}$ biodegradable COD). Feed to the Producer was diluted to obtain approximately 20 $g.l^{-1}$ total COD (ca. 8 $g.l^{-1}$ biodegradable COD), which corresponds with the average value of the pulp-mill effluent's total COD concentration.

3.4 Inoculum

All reactors were inoculated with fresh screen-dewatered monocultures of *A. fumigatus*, obtained from a microsieve reactor operated under predetermined optimum conditions. After inoculation the initial biomass concentration was approximately 4 $g.l^{-1}$ dry cells.

3.5 Operation

The temperature was automatically kept constant at the optimum of 45°C. Throughout the experiments the pH remained constant within ± 0,2 units and was therefore not automatically controlled. θ_c and τ_h were varied as shown in Table 9.

Table 9. θ_c and τ_h variations for the different bioreactors

BIOREACTOR	θ_c AND τ_h RANGE				STEP INCREASE (h)
	MIN (h)		MAX (h)		
	τ_h	θ_c	τ_h	θ_c	
CSTR without cell recycle	2	2	8,5	8,5	1
CSTR with cell recycle	2,5	4	3	19	1 to 2
CSTRs in series:					
Selector	2,8	12	2,8	12	None
Producer	2	2	10	10	1

To ensure that the theoretical and true data could be correlated for more than one biomass feed rate, two modes of operation were followed with the CSTRs in series. In the first mode all the harvested biomass of the Selector was supplied to the Producer, and in the second mode only half of the harvested biomass from the Selector was supplied to the Producer. Steady-state conditions were obtained and sampling took place as before.

3.6 Mathematic modelling and analytical methods

Theoretical biomass production and substrate utilisation for a particular set of operating parameters were calculated by using the Monod growth kinetic model (Appendix C) and applicable equations (Table 8) for each flow configuration. The theoretical values were then compared with corresponding analytical measurements. Borland Quattro Pro (Ver. 5) was used for all the statistical (regression) analyses and linearisations.

4. RESULTS, OBSERVATIONS AND DISCUSSION

4.1 Growth kinetic constants

The growth kinetic constants obtained for *A. fumigatus* grown on DSSL in optimum culture conditions are summarised in Table 10.

Table 10. Growth kinetic constants for *A. fumigatus*

PARAMETER	UNITS	VALUE
pH		5,5
Temperature	°C	45
Biodegradability of SSL	%	33,6
Maximum growth rate, μ_m	h^{-1}	0,318
Saturation constant, K_s	mg COD.l^{-1}	260
True growth yield, Y_g	g cells.g COD^{-1} utilised	0,70
Biomass decay rate, b	h^{-1}	0,016

The maximum growth rate (μ_m = 0,318 h^{-1}) and the high yield coefficient (Y_g = 0,7 g cells.g COD^{-1}) obtained with *A. fumigatus* exceed those of other known fungi. With *Geotrichum candidum*, for example, Kühn and Pretorius (1989a) obtained a μ_m of 0,26 h^{-1} and a Y_g of 0,38 g cells.g COD^{-1}, whereas Romantschuk and Lehtomäki (1978) obtained values of 0,28 h^{-1} and 0,55 g cells.g COD^{-1} respectively for *Paecilomyces varioti* grown on SSL.

It is especially the relatively high cell yield that is of interest for large-scale SCP. The amount of heat that has to removed from the biomass, because it is related to the cell yield (Equation 1), will be lower when *A. fumigatus* instead of, for example, *G. candidum* or *P. varioti* is used.

4.2 Producer (or CSTR without cell recycle)

Although the inoculum was virtually bacterial and yeast-free, excessive contamination with a corresponding loss of fungal biomass was observed at all the hydraulic residence times employed. Longer retention times resulted in faster contamination and *vice versa*.

The selection pressure exerted by the elevated temperature (45°C) alone was not enough to maintain a monoculture of *A. fumigatus* at the optimum pH for this fungus. After prolonged operation the fungus was essentially replaced, mainly by yeasts, at all the hydraulic retention times tested. The dominance of yeasts is most probably due to the high percentage of sugars (Table 2) in the biodegradable fraction of the feed.

If pulp-mill effluent is used as the feed for continuous cultivation of *A. fumigatus* in the Producer, contamination of the fungal biomass will take place with eventual loss of the fungal culture. This reactor set-up is therefore not suitable for cultivation of a monoculture of *A. fumigatus* on SSL in non-aseptic conditions.

4.3 Selector (or CSTR with cell recycle)

4.3.1 Monoculture growth. Within specific operating parameters, monoculture growth could be maintained with *A. fumigatus* in non-aseptic culture conditions

for an indefinite period. The following operating parameters need to be controlled:

Hydraulic retention time. This must be kept < 4 h. At longer τ_h the growth rate of smaller (non-filamentous) microorganisms was higher than the washout rate. This resulted in excessive contamination of the fungal culture by bacteria and yeasts. Fungal growth was progressively suppressed due to increased competition for the available substrate and the fungus was eventually lost.

The Selector is not restricted to a minimum τ_h. However, if τ_h is less than θ_{min} and problems are encountered with cell recycling, the reactor will behave as a simple CSTR would with a mean cell retention time equal to τ_h and washout will occur. For production plants this parameter should therefore, as a precautionary measure, be limited to a value not smaller than θ_{min} as expressed by the following equation (Grady & Lim, 1980):

$$\theta_{min} = \frac{K_s + S_o}{S_o(\mu_m - b) - K_s.b} \quad \ldots\ldots (9)$$

$$= 3{,}44 \text{ h}$$

For an assessment of SCP production in large-scale plants utilising this organism and a Selector, τ_h must be restricted to between 3,5 h and 4 h.

Biomass retention time. The number of filamentous species is limited by employing short retention times (θ_c = 1,5 to 2,5.τ_h - Chapter II, Section 4) and the Selector should therefore be operated with θ_c between 5,2 h (>1,5.θ_{min}) and 10 h (2,5.τ_h).

Biomass concentration. Because oxygen transfer is impaired at high biomass concentrations, the latter should be limited to concentrations where AO (Equation 2) is equal to or exceeds r_o inside the reactor, to prevent oxygen from becoming the limiting substrate. In mathematical terms this constraint can be expressed as:

$$AO - r_o \geq 0 \qquad \text{..... (10)}$$

4.3.2 Experimental data. A good resemblance was found to exist between the practical and the theoretical data. Typical steady-state data obtained with the Selector and corresponding calculated values are graphically presented in Figure 8.

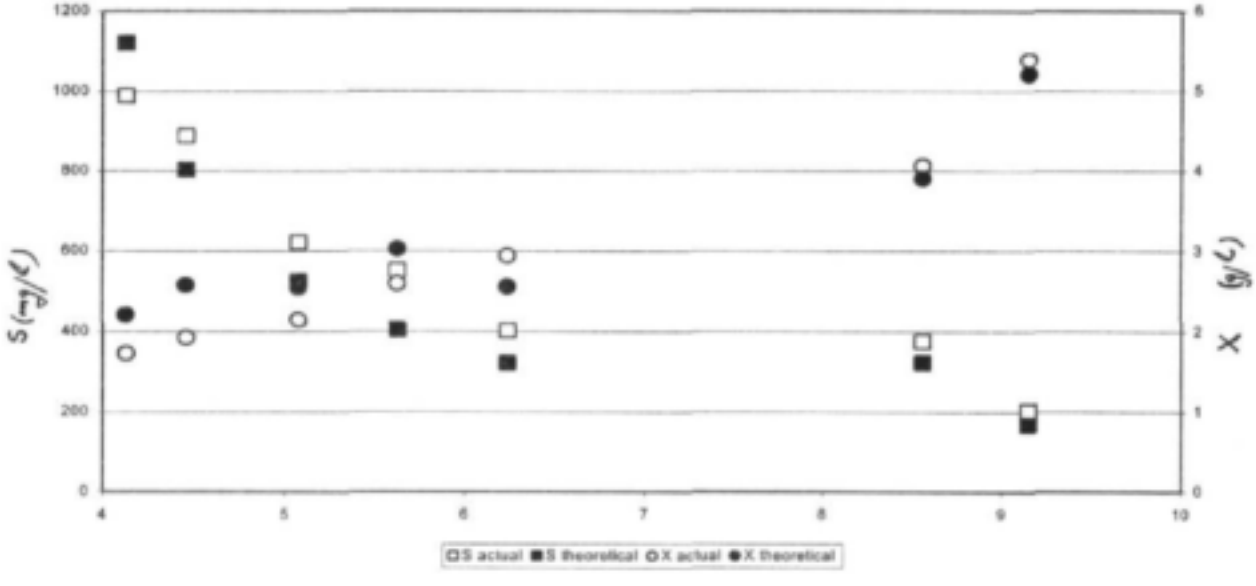


Figure 8. Performance in a Selector with varying cell age (θ_c)

The variance (R^2) between actual (measured) and theoretical (calculated) data obtained in a regression analysis was found to be 0,83 for S and 0,99 for X. In all but one case the Monod model predicted lower values for S than those analysed in the effluent of the laboratory set-up. The higher values for S obtained in the experimental reactor could possibly be attributed to refractory by-product generation during microbial metabolism (Chapter III, Section 2).

In industries employing process technology a figure of between 10% and 20% conformance between theoretically predicted and practically obtained yields is generally accepted (Bailey & Ollis, 1986). Within this constraint, the Monod model could be used to predict X with high accuracy and S with reasonable accuracy for a Selector as used in the laboratory study.

4.4 CSTRs in series

With a Selector/Producer set-up, it was possible to maintain a monoculture of the fungus indefinitely if the τ_h of the Producer was kept below 8 h. This was achieved when the full as well as half the harvest flow from the Selector was fed to the Producer. When the τ_h in the producer was increased to more than 8 h, excessive contamination with unwanted microorganisms, as has been observed in the single Producer, occurred. Typical operating data for the experimental CSTRs in series are presented in Table 11.

Table 11. Performance of CSTRs in series

REACTOR	τ_h (h)	θ_c (h)	S_o (g COD l^{-1})	S*	S**	X* (g biomass.l-1)	X**	r_o (g.(l.h)$^{-1}$)
Selector reactor	2,78	9,15	2,98	0,51	0,17	5,42	5,65	1,01
Producer reactor								
(a) Inflow - full	1,95	1,95	6,34	5,71	5,32	0,46	0,72	0,31
harvest from	2,87	2,87	6,34	4,24	4,27	1,40	1,26	0,54
Selector	3,05	3,05	6,32	4,09	3,36	1,43	1,81	0,76
	4,12	4,12	6,04	0,53	0,72	4,28	3,50	1,17
	6,14	6,14	5,97	0,21	0,25	4,37	4,30	0,96
(b) Inflow - half	7,09	7,09	7,11	0,24	0,25	4,38	4,31	0,95
harvest from	7,52	7,52	6,40	0,17	0,19	4,34	4,22	0,81
Selector	8,74	8,74	6,14	0,14	0,13	4,30	4,01	0,61
	9,81	9,81	7,03	0,12	0,12	4,78	4,08	0,60

* Calculated (theoretical) value

** Actual (measured) value

Typical steady-state data for X and S, obtained with the experimental series reactor set-up, and corresponding calculated values are graphically presented in Figure 9. The variance between the measured and theoretical biomass concentration was 0,95 and the corresponding value for the substrate concentration was 0,99. This indicates that the Monod model can be used to predict X and S with acceptable accuracy.

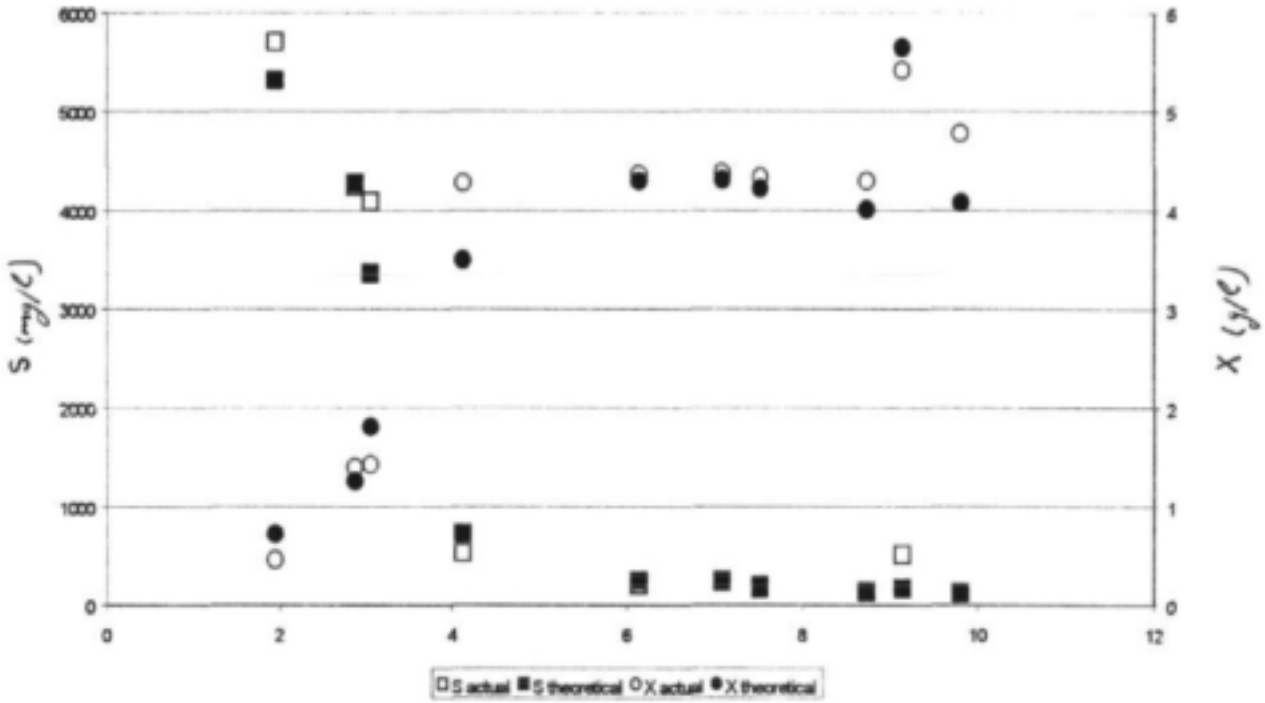


Figure 9. Theoretical vs actual values for X and S obtained in CSTRs in series

The measured biomass concentration in the Producer increased sharply from approximately 1,4 to 4,3 $g.l^{-1}$ (dry cells) when the τ_h in this reactor was increased from 3,05 to 4,12 h (Table 11). As could be expected, the effluent substrate concentration in the Producer accordingly declined from 4,09 to 0,53 g $COD.l^{-1}$ (Table 12). This substantial increase in the substrate removal over a relatively small increase in the hydraulic retention time could be explained by the point of washout (Grady & Lim, 1980) for a single CSTR without cell recycle.

Washout in this reactor, which is defined by Equation 9 with θ_c replaced by τ_h, would occur when $\tau_h < 3,5$ h, utilising a feed concentration of approximately 6,3 $g.l^{-1}$ biodegradable COD, as in the above study. In the Selector/Producer set-up, washout in the Producer is prevented because fresh cells are constantly added to the influent stream (from the Selector). Reducing τ_h in the Producer would result in the cell concentration in this reactor approaching that in the influent. Although the organisms would be growing and removing substrate at a rapid specific rate in this instance, the time for reaction is not long enough to achieve substantial substrate removal. To utilise more of the available substrate, a minimum $\tau_h = 4$ h will be used for the Producer.

The upper and lower constraints for τ_h for the Producer in a Selector/Producer set-up will be set as 4 h and 8 h respectively.

5. SUMMARY OF OPERATING PARAMETERS AND CONSTRAINTS

The previous findings are summarised in Table 12. The theoretical values can be used with reasonable accuracy for estimates where growth kinetic parameters or constraints to growing *A. fumigatus* on a large scale in a Selector or Selector/Producer reactor chain are employed.

Table 12. Growth kinetic constants and constraints for growing *A. fumigatus* on a large scale with DSSL as substrate

PARAMETER	UNITS	VALUE	
Growth-related			
pH		5,5	
Temperature	°C	45	
Biodegradability of SSL	%	33,6	
Maximum growth rate, μ_m	h^{-1}	0,318	
Saturation constant, K_s	g COD.l^{-1}	0,260	
True growth yield, Y_g	g cells.g COD^{-1} utilised	0,700	
Biomass decay rate, b	h^{-1}	0,016	
		Min	Max
Operation-related			
(a) Producer reactor			
τ_h	h^{-1}	4	8
r_o	g.(l.h)$^{-1}$	-	OA*
S	g COD.l^{-1}	0	1
S_o	g COD.l^{-1}	0	7,20
X (dry cells)	g.l^{-1}	-	10
(b) Selector reactor			
τ_h	h^{-1}	θ_{min}	4
θ_c	h^{-1}	5,2	10
r_o	g.(l.h)$^{-1}$	-	OA*
S	g COD.l^{-1}	0	1
S_o	g COD.l^{-1}	0	7,20
X (dry cells)	g.l^{-1}	-	10

* $AO = 1,491.\ e^{(-0,1951.X)}$ g O_2.(l.h)$^{-1}$

6. CONCLUSIONS

The following conclusions may be drawn from the biomass production studies:

- *A. fumigatus*, when grown on SSL, has a relatively high yield coefficient which can contribute favourably to the economics of a large-scale SCP production plant. Cooling requirements and oxygen demand during the aerobic exothermic fermentation reaction will be lower than for other microorganisms with a lower yield coefficient.

- The ability of *A. fumigatus* to grow at higher than ambient temperatures does not suffice as the only selective pressure to sustain monoculture growth under non-aseptic culture conditions. The single CSTR without recycle, such as the Producer reactor, is not suitable for continuous fungal biomass production.

- Dilution of the pulp-mill effluent, if used as a substrate for SCP production, is not required in a CSTR without cell recycle, but the fungal culture is prone to excessive contamination, mainly by yeasts, resulting in the complete loss of the fungal culture with prolonged operation.

- A CSTR with a microsieve as cell separator, such as the Selector reactor, could indefinitely maintain a monoculture of *A. fumigatus* on DSSL. Dilution of the feed will be required to utilise a strong organic effluent in such a flow configuration.

- By combining a Selector and a Producer in a series flow arrangement, a stable process could be established for maintaining monoculture growth of

A. fumigatus in non-aseptic culture conditions with minimum dilution requirements.

- Practical (lower and upper) limits for the operating parameters of a Selector and CSTRs in series flow configuration could be established (Table 12).

- Monod growth kinetics could be applied with accuracy for modelling and upscaling of the Selector reactor and CSTRs in series.

To predict reactor performance and estimate SCP production in large-scale plants utilising the mentioned flow configurations, a better understanding is required of system behaviour for varying operating parameters. This aspect is addressed in the next chapter.

CHAPTER VI

SYSTEM BEHAVIOUR

1. INTRODUCTION

Laboratory studies revealed that SSL may be used as a feed for continuous growth of *Aspergillus fumigatus* as a monoculture in non-aseptic culture conditions. This can be achieved by utilising a Selector reactor or CSTRs in series flow configuration.

To preselect the most appropriate of the above-mentioned flow configurations for large-scale SCP production requires a better understanding of the influence varying operating parameters can have on a system. In this chapter system behaviour of the Selector reactor and CSTRs in series flow configuration is discussed.

An assessment was made of the theoretical amount of SCP that can be produced from the pulp-mill effluent, utilising different flow configurations. For this, the optimum operation of a Selector reactor and CSTRs in series flow configuration was determined with standard computer (spreadsheet) software.

2. BACKGROUND

2.1 Unit processes

Atkinson and Mavituna (1983), as a general rule, recommend selecting a process that requires the minimum number of unit processes and stages. More stages require greater capital outlay and usually increase the running costs. Measured against this criterion, the Selector reactor would be more suitable than CSTRs in series.

2.2 Operating parameters

Different reactor systems have different dynamic operating parameters, and these can be adjusted to improve operation and/or productivity. Kühn and Pretorius (1989b) have for example shown how biomass production increased from 0,39 to 0,53 g.(l.h)$^{-1}$ and how COD reduction decreased from 49% to 40% when the cell retention time was decreased from 18 h to 10 h. This was achieved in a microsieve process utilising DSSL as the substrate to grow *Geotrichum candidum*. The influence of varying operating parameters for the different flow configurations is described in this study.

2.3 Limiting utilities

The basic utilities that have to be taken into account for cultivating *A. fumigatus* in the mentioned reactor configurations are oxygen and dilution water.

Oxygen. This has been identified as the limiting substrate (Wille, 1992) in the Selector reactor when operating at high biomass concentrations. Oxygen availability as defined by Equation 2 will determine the maximum biomass concentration that can be maintained in a reactor.

Dilution water. A serious drawback of the Selector reactor when the feed contains high concentrations of biodegradable organic matter, is the necessity to dilute the feed. This is required to prevent oxygen from becoming the limiting substrate.

Dilution could be achieved by adding potable water or by reintroducing the effluent of the reactor into the feed stream. Both options would increase the operating costs and should be minimised for economic reasons.

Because water is not in abundance in South Africa (Department of Water Affairs, 1986), utilising effluent from the reactor to dilute the feed would be the most appropriate method to achieve the required low feed concentration. To prevent reintroducing large numbers of unwanted microorganisms into the reactor, sterilisation and subsequent cooling of the recycled effluent will be necessary.

Excess heat (steam) is produced during the pulping process at most pulp mills that facilitate chemical recovery (Water Research Commission, 1990). Sterilisation, if required, can therefore be achieved at little extra cost. Although this was also the situation at the pulp mill (Thubron, 1991) whose effluent was utilised in this study, it may not generally apply to all pulp mills.

The detrimental effect on the economics of feed dilution can be minimised if a Selector/Producer configuration instead of a single Selector reactor is used, because the series reactor configuration requires less feed dilution.

From the above follows that, for the particular mill, oxygen (air) supply to the reactors will be the single most important utility for growing *A. fumigatus*. To improve the economics of a full-scale SCP production plant, dilution of the feed should be minimised. These aspects were considered during the following system behaviour studies.

2.4 Feed concentration

The ever increasing pressure on industry in South Africa to reduce water consumption has resulted in waste streams becoming increasingly concentrated and this trend could be expected to continue in future (Murray, 1987). This will place more emphasis on the economics of feed dilution when the most appropriate process, a Selector or CSTRs in series flow configuration, has to be selected. A possible future increase in COD concentration of the pulp mill's effluent was also taken into account in these studies.

3. MATERIALS AND METHODS

Monod (1949) growth kinetics and reactor modelling (Grady & Lim, 1980), which were found to describe the experimentally obtained data with good accuracy (Chapter V), were used in the system behaviour studies. The following flow systems were theoretically analysed:

- CSTR with and without cell recycle (individually). Theoretical data were generated by applying the equations listed in Table 8 (Chapter V) and growth kinetic constants from Table 13 (Chapter V) to the Producer and Selector reactors (separately).

- CSTR with cell recycle and CSTRs in series reactors. Utilising the pulp mill's effluent and constraints identified for the different (single) reactors, optimum operating conditions were determined for each configuration with:

  (a) maximum oxygen utilisation (because air/oxygen supply to the reactor represents the single most expensive item for each reactor system);

  (b) minimum dilution of the feed to the Selector reactor for situations where dilution could be more expensive than air supply.

Quattro Pro's (Ver. 5.0) spreadsheet and its standard Optimizer function were used for the optimisation. Table 13 summarises the independent variables for each configuration.

Table 13. Independent variables for optimising the Selector and CSTRs in Series reactors

REACTOR	INDEPENDENT VARIABLES
Selector reactor	θ_c, τ_h, S_o
Selector/Producer reactors	θ_c, τ_h, S_o, F_1, F_{o2}, V_2

A schematic representation of the Selector and CSTRs in Series flow configuration that was analysed is shown in Figures 10 and 11 respectively.

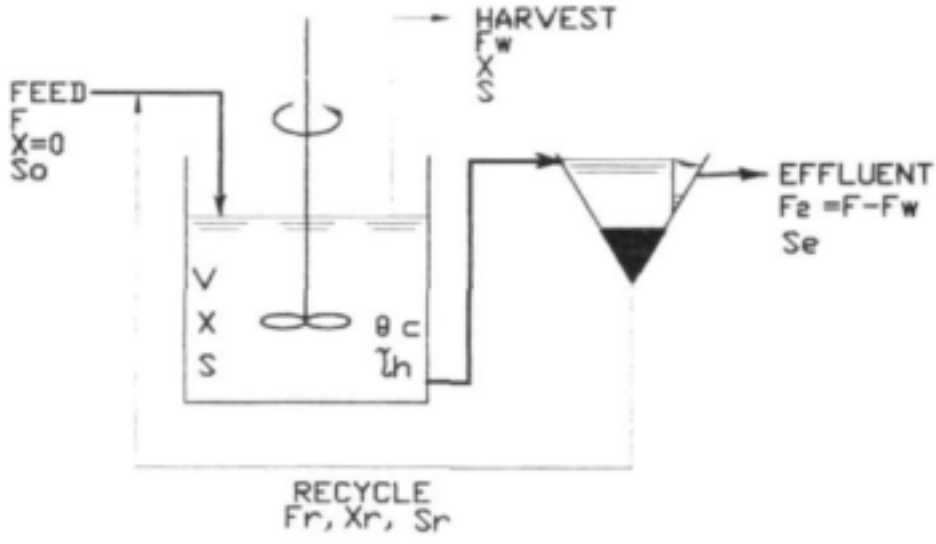


Figure 10. Schematic representation of Selector reactor

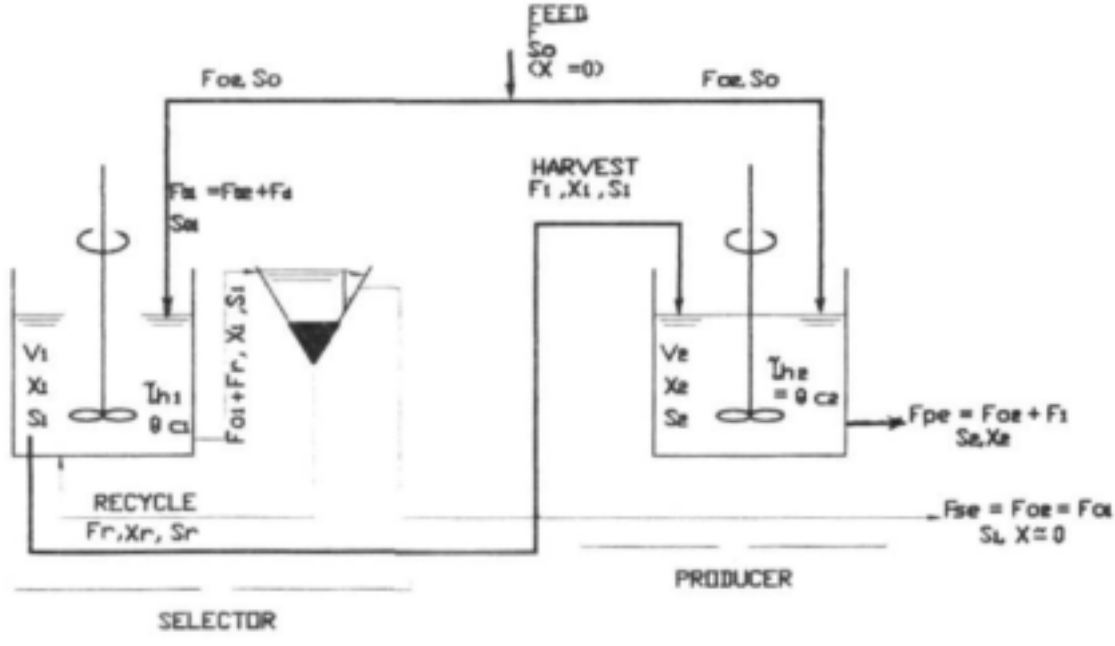


Figure 11. Schematic representation of CSTRs in series

4. RESULTS, OBSERVATIONS AND DISCUSSION

4.1 Producer and Selector reactors (CSTR without and with cell recycle)

Table 14 reflects data generated for the Producer and Selector reactors under different operating conditions. The following observations are made from system behaviour data (Table 14) when the reactors were operated within the constraints given in Table 12:

Washout in the Producer (A i)): Biomass production (P) and substrate removal (COD Red.) increase sharply when τ_h is increased from 3,45 h to 3,5 h to 4,0 h. Thereafter productivity declines and substrate removal increases moderately when τ_h is operated within the recommended (Table 12) upper and lower limits ($4 \leq \tau_h \leq 8$).

Oxygen as limiting substrate. Whereas the Producer could be operated comfortably within the recommended constraints (A ii)), this was not the case for the Selector. At minimum τ_h (= 3,5 h), oxygen starvation was observed at θ_c = 5,55 h (B i)). The latter could be increased to 6,65 h at maximum τ_h before oxygen became the limiting substrate (B ii)). Dilution of the feed will be required to operate at a higher θ_c.

Maximum feed concentration. The maximum substrate concentration that can be introduced into the different flow configurations when the reactors are run within the established constraints is 11,8 g biodegradable COD.l^{-1} (at τ_h = 8 h) in the Producer (C i)) and 7,2 g biodegradable COD.l^{-1} (at τ_h = 3,5 h and θ_c = 5,2) in the Selector (C ii)).

Table 14. System behaviour of the Producer and Selector reactor

OPERATION	τ_h (h)	θ_c (h)	S_0 (g COD.l^{-1})	S (g COD.l^{-1})	X (g.l^{-1})	OA (g O_2.(l. h)$^{-1}$)	r_O (g O_2.(l. h)$^{-1}$)	OA-r_O (g O_2.(l. h)$^{-1}$)	P** (g (l. h)$^{-1}$)	COD (g.l. $^{-1}$)
A Producer										
i) Washout	3,45		7,20	7,20	0,00	1,200	0,000	1,200	0,000	0,00
	3,50		7,20	4,82	1,32	1,154	0,116	1,038	0,376	1,98
ii) At $4 \leq \tau_h \leq 8$	4,00		7,20	1,33	3,60	0,739	0,288	0,451	0,900	5,47
	5,00		7,20	0,55	4,05	0,677	0,278	0,399	0,810	6,25
	6,00		7,20	0,35	4,12	0,668	0,251	0,416	0,686	6,45
	7,00		7,20	0,26	4,12	0,668	0,229	0,439	0,588	6,54
	8,00		7,20	0,21	4,09	0,671	0,210	0,461	0,511	6,59
B Selector										
i) At min τ_h	3,50	5,20	7,20	0,49	6,06	0,458	0,405	0,053	1,730	6,31
	3,50	5,55	7,20	0,42	6,51	0,418	0,418	0,000	1,859	6,65
	3,50	6,00	7,20	(0,35)	(7,06)	0,376	0,430	-0,054*	-	-
ii) At max τ_h	4,00	5,20	7,20	0,49	5,30	0,530	0,354	0,176	1,324	6,31
	4,00	6,00	7,20	0,35	6,18	0,447	0,377	0,070	1,545	6,45
	4,00	6,65	7,20	0,29	6,85	0,392	0,392	0,000	1,713	6,51
	4,00	7,00	7,20	(0,26)	(7,21)	0,366	0,400	-0,034*	-	-
C At max feed										
i) Producer	4,00		10,3	1,33	5,90	0,472	0,472	0	1,474	8,97
	8,00		11,8	0,21	7,16	0,369	0,369	0	0,895	11,59
ii) Selector	3,50	5,2	7,20	0,49	6,39	0,428	0,428	0	1,826	6,71
	3,50	10,0	4,50	0,15	7,51	0,344	0,344	0	2,143	4,35
	4,00	5,2	8,10	0,49	6,39	0,428	0,429	0	1,596	7,61
	4,00	10,0	5,10	0,15	7,51	0,343	0,344	0	1,878	4,95

* Negative value indicates insufficient oxygen present

P** = Biomass production rate (g (l.h)$^{-1}$)

Values in brackets indicate hypothetical case - because oxygen is limiting these values cannot be achieved

The above biodegradable values (11,8 g and 7,2 g COD.l $^{-1)}$ would conform to a total COD of approximately 35 g.l $^{-1}$ and 21 g.l $^{-1}$ SSL respectively if *A. fumigatus* is employed as the microorganism to utilise the pulp-mill effluent. Because this effluent could reach total COD values as high as 26 g.l $^{-1}$ (Thubron, 1991), the Producer but not the Selector could be employed without dilution of the feed.

For large-scale SCP production utilising the pulp mill's effluent, dilution of the feed or a storage facility for concentration equalisation will be required if a Selector reactor is used to grow *A. fumigatus*.

4.2 CSTR with cell recycle (Selector) and CSTRs in series (Selector/Producer)

4.2.1 Selector reactor. Table 15 shows the optimum operating parameters for continuously growing *A. fumigatus* in a Selector reactor utilising pulp-mill effluent under conditions of:

a) optimal oxygen utilisation,

b) minimum feed dilution, and

c) maximum substrate concentration that can be fed into the reactor without oxygen depletion.

Table 15. Optimum and oxygen-limiting parameters for the Selector reactor

OPERATION	τ_h (h)	θ_C (h)	S_o (g COD. l^{-1})	S (g COD. l^{-1})	X ($g\ l^{-1}$)	OA (g O_2. $(l\ h)^{-1}$)	r_s (g O_2. $(l\ h)^{-1}$)	Fd* ($m^3.h^{-1}$)	Ft* ($m^3.h^{-1}$)	V (m^3)	RO ($t\ O_2\ h^{-1}$)
a) Oxygen max.	3,5	10,0	4,5	0,15	7,52	0,344	0,344	1 700	5 040	16 640	5,70
b) Dilution min.	4,0	6,6	6,8	0,29	6,85	0,393	0,392	0	3 335	13 340	5,23
c) Max. feed con	4,0	6,0	7,2	0,35	6,66	0,407	0,407	0	3 335	13 340	5,43

* Fd = Dilution requirements (no substrate in this stream)

** Ft = Total feed, including dilution water (if applicable)

The above table shows the effect of different starting points with regard to optimal operating conditions in a Selector reactor. To operate the Selector reactor at maximum oxygen utilisation (RO) requires a dilution stream of 1 700 $m^3.h^{-1}$ because S_o must be kept at 4,5 g COD.l^{-1} (Table 15 a). This will increase the total inflow to the reactor by more than 51%, from 3 335 $m^3.h^{-1}$ to 5 040 $m^3.h^{-1}$, so that more extensive process equipment will be required compared to a system where no feed dilution is maintained (Table 15 b or c). When feed dilution is maintained, the total reactor volume (V = 17 640 m^3) will be roughly a third larger than in a system without feed dilution (V = 13 340 m^3). The oxygen demand will be 14% less if the feed is diluted, compared to a similar process operated at minimum (no) dilution.

The importance of carefully selecting operating parameters to run the system under specific desirable conditions is also evident from the data in Table 15. The feed to the microsieve reactor need not be diluted in order to prevent oxygen from becoming the limiting substrate. Instead the system could be operated at shorter θ_c (6,65 h instead of 10 h). This results in the reactor biomass concentration being lower (X = 6,84 g instead of 7,52 g.l^{-1} dry cells) and, because oxygen availability inside the reactor is now higher (OA = 0,392 g instead of 0,344 g $O_2.(l.h)^{-1}$), the reactor can be fed the full-strength pulp-mill effluent.

The limiting biodegradable feed concentration that can be utilised by *A. fumigatus* in this set-up (without feed dilution) was found to be 7,2 g COD.l^{-1} (Table 15c), which corresponds to a total feed COD of 21,5 g COD.l^{-1}.

An increase in the biodegradable feed concentration, as could be expected in future (Section 2.4), would increase the dilution requirements significantly. For example, a 25% increase in the biodegradable COD concentration in the feed stream will require an added 64% (based on the volume of the feed) dilution.

4.2.2 Selector/Producer series reactors. Table 16 shows the optimum operating parameters for continuously growing *A. fumigatus* in a Selector/Producer series reactor configuration under conditions of:

a) optimal oxygen utilisation and

b) minimum feed dilution.

The symbols used conform to the schematic representation of CSTRs in series arrangement (Figure 11), with subscripts 1 and 2 referring to the Selector and Producer reactors respectively.

Table 16. Operational parameters for a Selector/Producer in series reactor

SELECTOR REACTOR										
OPTIMUM OPERATION	τ_{h1} (h)	θ_{C1} (h)	S_{s1} (g COD.l^{-1})	S_1	X_1 (g.l^{-1})	$OA_1=r_{o1}$ (**)	F_{O1} ($m^3.h^{-1}$)	Fd*	V_1 (m^3)	F_{se} ($m^3.h^{-1}$)
a) Oxygen max.	3,75	10	4,8	0,15	7,52	0,344	1 255	360	4 700	415
b) Dilution min.	4	6,65	6,8	0,29	6,65	0,392	3 335	0	13 340	3 335
PRODUCER REACTOR										
OPTIMUM OPERATION	τ_{h2} (h)	F_{O2} ($m^3.h^{-1}$)	F_{O2}	S_2 (g.l^{-1})	X_2 (g.l^{-1})	OA_2 (g O_2. (l. h)$^{-1}$)	r_{O2}	F_1 ($m^3.h^{-1}$)	V_2 (m^3)	RO_t*** (t $O_2.h^{-1}$)
a) Oxygen max.	8	2 450	885	0,13	4,54	0,615	0,196	360	23 500	6,270
b) Dilution min.	0	0	0	0	0	0	0	0	0	5,230

* Fd = Dilution requirements (no substrate or biomass in this stream)

** g O_2.(l.h)$^{-1}$

*** $RO_t = RO_1 + RO_2$

It follows from the above table that the optimum operation for CSTRs in series with maximum utilisation of the available oxygen would be in a Selector/Producer set-up according to data set 16a). A single Selector reactor with operating parameters according to data set 16b) could be used to minimise feed dilution.

Oxygen utilisation will be higher in the CSTRs in series configuration (Table 16: RO_t = 6,27 t $O_2.h^{-1}$) than in the Selector reactor (Table 15: RO_t = 5,70 t $O_2.h^{-1}$) when both systems are run with maximum oxygen utilisation.

As observed before (Section 4.1), the pulp mill's effluent can be utilised as a feed without dilution when a Selector reactor (only) is used. This would not generally be the case for any substrate. For example, if the biodegradable substrate concentration is higher than 7,2 g COD.l $^{-1}$, *oxygen will become the limiting substrate (Table 15c). Feed dilution will* then be required to prevent oxygen from becoming the limiting substrate.

A comparison of the data in Tables 15 and 16 shows that oxygen is better utilised in a Selector/Producer in series reactor configuration than in a Selector reactor (RO_t = 6,27 t *vs* RO = 5,70 t $O_2.h^{-1}$).

The dilution requirements decreased drastically (almost 79%) from 1 700 to 360 $m^3.h^{-1}$ in a Selector/Producer configuration, compared to a single Selector reactor. Thus, by employing a Selector/Producer set-up instead of a single Selector reactor, the dilution requirements can be decreased by almost 79% without increasing the air requirements. This aspect is important in the design of full-scale plants. If the air supply is more expensive than the feed dilution, a Selector/Producer in series reactor configuration would be more economical than a single Selector reactor.

An increase in the biodegradable feed concentration, as discussed in Section 2.4, would have less impact on the dilution requirements in the Selector/Producer in series reactor configuration than in the Selector (only) reactor. For example, a 25% increase in the biodegradable COD concentration in the feed stream will require an added 14% (based on the volume of the feed) dilution in the Selector/Producer in series reactor configuration compared to a corresponding figure of 64% in the Selector reactor. This constitutes a saving of 50% in the dilution requirements for the series reactor configuration.

4.3 SCP production

The potential of the total pulp-mill effluent stream as a substrate for SCP production in the different reactor configurations at optimum operating conditions can be estimated from the data in Tables 15 and 16. The theoretical biomass production, based on the optimum operating parameters for maximum utilisation of the available oxygen, is approximately the same for the different reactor configurations: In the Selector reactor 13,28 $t.h^{-1}$ of dry cells will be produced whereas the corresponding figure for the Selector/Producer configuration is 12,76 $t.h^{-1}$ (4% less). When minimum dilution is required, a (single) Selector reactor could be used and biomass production would be 13,74 $t.h^{-1}$. This is 3,5% higher than can be achieved with the same process when the available oxygen is optimally utilised.

The above biomass production figures, which were obtained for the different reactor configurations, are very similar and other factors not considered in this study will have to be taken into account before deciding on the reactor configuration that would best suit this particular case.

A CSTR with recycle such as the microsieve process could be utilised as a continuous production process for the large-scale cultivation of fungal protein, utilising the full stream of pulp-mill effluent as the substrate (flow = 80 $Ml.d^{-1}$; biodegradable COD = 6,8 $g.l^{-1}$). SCP production in such a process would yield approximately 13,74 $t.h^{-1}$ or 120 000 t per year of SCP. This conforms to 30% of South Africa's estimated total feed protein deficit for the year 2000 (Cloete, 1990).

5. CONCLUSIONS

The following conclusions could be drawn from the optimisation studies:

- For optimum utilisation of the available oxygen, the dilution requirements could be reduced by 79% if a Selector/Producer series configuration instead of a single CSTR with recycle (Selector reactor) is used.

- The dilution requirements for a Selector/Producer in series reactor set-up are 50% less than for a Selector (only) reactor if the biodegradable concentration of the feed, as used in this study, is increased by 25%.

- The theoretical biomass production is 4% lower in a Selector/Producer set-up than in a CSTR with recycle.

- The microsieve process with minimum dilution could be used to produce SCP utilising the pulp-mill effluent as a substrate and *A. fumigatus* as the fungus. Maximum biomass production was achieved with this configuration.

- The maximum theoretical SCP production utilising the pulp-mill effluent as a substrate would be obtained when a CSTR with recycle such as the microsieve process is used and would total approximately 120 000 $t.a^{-1}$. This is approximately 30% of South Africa's estimated total feed protein deficit for the year 2000 (Cloete, 1990).

CHAPTER VII

SUMMARY AND RECOMMENDATIONS

1. SUMMARY

1.1 South Africa will have to augment its agriculturally produced protein to feed its ever increasing population. Since a global shortfall of protein can be expected to occur early in the next century, SCP production should be considered to relief food shortages.

1.2 Pulp-mill effluent was identified as a high-strength organic waste stream that can be utilised as a substrate for SCP production.

1.3 The microsieve process was identified as the most suitable process for large-scale SCP production under continuous, non-aseptic culture conditions.

1.4 For commercial application three major drawbacks were identified with the microsieve process as operated at that stage:

- Mesophilic fungi were employed that require extensive cooling to maintain optimum growth temperatures.

- It is difficult to maintain a monoculture due to excessive contamination by bacteria and yeasts at the low operating temperatures.

- Dilution of high substrate concentrations is needed to prevent oxygen from becoming the limiting substrate.

1.5 The pulp-mill effluent's biodegradable COD was found to be approximately 40% of the total COD, which translates into 234 000 $t.a^{-1}$ COD that will be available as substrate COD for SCP production.

1.6 A thermotolerant fungus that can utilise DSSL as food, *Aspergillus fumigatus*, could be isolated from a mixed culture using the selection pressure technique (Pretorius, 1987). The fungus was found to have optimum growth at a temperature of 45°C and a pH between 5,25 and 5,75.

1.7 *Aspergillus fumigatus* was able to utilise only 33,6% of the pulp mill's total COD concentration.

1.8 Growth kinetic constants for *Aspergillus fumigatus* were determined in a laboratory study and are shown in Table 17.

Table 17. Growth kinetic constants for *A. fumigatus*

PARAMETER	UNITS	VALUE
pH		5,5
Temperature	°C	45
Biodegradability of SSL	%	33,6
Maximum growth rate, μ_m	h^{-1}	0,318
Saturation constant, K_s	mg COD.l $^{-1}$	260
True growth yield, Y_g	g cells.g COD^{-1} utilised	0,70
Biomass decay rate, b	h^{-1}	0,016

1.9 The amino-acid composition of *A. fumigatus* compares well with other protein sources generally used as feed for animals. A slightly higher concentration of the essential amino acids lysine and methionine renders the SCP from this fungus superior to SCP from *Geotrichum* sp.

1.10 The elevated operating temperature exerted additional selection pressure on the biological system and it was possible to maintain monoculture growth of *A. fumigatus* indefinitely in a microsieve reactor.

1.11 Stable monoculture growth in non-aseptic conditions could only be maintained in a Selector reactor and a Selector/Producer in series reactor configuration, but not in a single CSTR without cell recycle.

1.12 Theoretically predicted reactor performance utilising Monod growth kinetics and reactor modelling resembled actual performance in a laboratory set-up.

1.13 System behaviour for a Selector and Selector/Producer in series reactor configuration was studied and optimum performance of the two configurations was determined.

1.14 *Aspergillus fumigatus* could be grown continuously by using the specific pulp mill's average effluent stream in a Selector reactor without requiring dilution of the feed.

1.15 Optimum oxygen utilisation required dilution of the feed in both the microsieve and the Selector/Producer configurations. With the latter configuration 75% less dilution was needed. Biomass production was 4% lower in the Selector/Producer reactors than in the microsieve reactor.

1.16 Maximum theoretical SCP production utilising the pulp-mill effluent as substrate (flow = 80 000 $m^3.d^{-1}$; biodegradable COD = 6,8 $g.l^{-1}$) and *Aspergillus fumigatus* as fungus can be obtained in a microsieve process and would total approximately 120 000 t per annum. This figure represents 30% of South Africa's estimated total feed protein deficit in the year 2000.

2. RECOMMENDATIONS

The study that was undertaken under the heading *Mycoprotein production on spent sulphite liquor* opened up a completely new field for research, namely the continuous growth of fungi at thermophilic temperatures as fully submerged cultures. This research can be used for:

2.1 Mycotoxigenic studies. Extensive feeding, toxicity and mutability studies with *Aspergillus fumigatus* should be undertaken to ensure its safe use as SCP.

2.2 Pilot studies. A pilot plant for SCP production with the proposed process at thermophilic temperatures and using the fungus *Aspergillus fumigatus* is required to determine whether further development into a full-scale production process is justifiable.

2.3 Production of microbial by-products. The use of *Aspergillus fumigatus* for the possible production of valuable by-products should be investigated.

2.4 Mycoprotein production at elevated temperatures. Organic effluent that is discharged in large quantities at an elevated temperature (Chapter I) by other "wet" industries in South Africa can be assessed for its value as a substrate to produce SCP.

2.5 Biological effluent treatment at elevated temperatures. Maximising the COD removal from biological effluents at elevated temperatures instead of mycoprotein production could be investigated. Effluent treatment was not considered in this study.

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APPENDIX A

SCP PRODUCTION:

SUMMARY OF LITERATURE SURVEY

LITERATURE SURVEY-MISCELLANEOUS SCP PROCESSES (SOLOMONS, 1983)

MICRO ORGANISM	PROCESS, EMPLOYER OR PLACE	SUBSTRATE	MICROORGANISM EMPLOYED	PLANT SIZE	PLANT SCALE	PRODUCT
PURE SUBSTANCES AS SUBSTRATE						
Yeast	BP - Lavera	wax	*Candida lipolitica*	16000 t/a	comm	Animal fodder
Yeast	BP-Dainippon	purified paraffin	*Candida pichia*	60000 t/a	comm	Animal fodder
Yeast	BP-Grangemouth	purified paraffin		4000 t/a	demo	Animal fodder
Yeast	BP-Grangemouth	purified paraffin		25 t/a	pilot	Animal fodder
Yeast	BP-Liquichimica	purified paraffin	*Candida novellus*	100000 t/a	comm	Animal fodder
Bacteria	Esso-Nestle	purified paraffin		t/a	demo	Animal fodder
Yeast	BP/ANIC-Sarroch	purified paraffin		100000 t/a	comm	Animal fodder
Bacteria	Hoechst	pure methanol	*Methylomonas clara*	2000 t/a	pilot	Food yeast
Bacteria	ICI-Prutreen	pure methanol	*Methylophilus methylotrophus*	60000 t/a	comm	Animal fodder
Bacteria	ICI-Prutreen	pure methanol	*Methylophilus methylotrophus*	1000 t/a	pilot	Animal fodder
Yeast	Mitsubishi Gas Chemical Co	pure methanol	*Kloeckera gen.*	2 l	research	Animal fodder
Bacteria	Shell	pure methane	*2-10 organisms*	t/a	demo	Animal fodder
Yeast	Mitsubishi Petrochem Co	pure ethanol	*C. ethanothermophilum*	100 t/a	pilot	Animal fodder
Yeast	Mitsubishi Petrochem Co	pure ethanol	*C. acidothermophilum*	100000 t/a	comm	Animal fodder
Yeast	Garrido-Schick Quorn	pure ethanol	*H. anomela*	100 t/a	pilot	Animal fodder

AGRICULTURAL AND INDUSTRIAL WASTES AS SUBSTRATE:

MICROORGM	PROCESS, EMPLOYER OR PLACE	SUBSTRATE	SOURCE	MICROORGANISM EMPLOYED	PLANT SIZE	PLANT SCALE	PRODUCT	REFERENCE
Yeast	Shanghai Brewing Sub-contractor Res Inst	Monosodium glutamate	production w/water	*Candida tropicalis*	30 l	research	Animal fodder	Yhao, 1988
Fungi	Univ of Pretoria	Monocarboxylic acid	synthetic fuel	*Geotrichum candidum*	13.5 l	research	Animal fodder	Kilian, 1989
Yeast	Tate and Lyle	Confectionery effl	sweet factory	*Candida utilis*	500 t/a	comm	Animal fodder	Solomons, 1983
Fungi	CIAT	Cassava	starch industry	*Aspergillus fumigatus I-21A*	3000 l	pilot	Animal fodder	Gregory *et al.*, 1976; Santos & Gómez, 1993
Yeast	Waldhof - Inst. F. G?rungsw.	Beet mol; wood sugar	industrial effl	*Candida utilis*	15000 t/a	comm	Food yeast	Andersen, 1979
Yeast	Amber Laboratoriese	Whey	dairy industry	*Saccharomyces fragilis*	5000 t/a	comm	Food yeast/ethanol	Solomons, 1983
Yeast	Knudson Creamery Co	Whey	dary industry	*Saccharomyces fragilis*	750 t/a	comm	Food yeast	Solomons, 1983
Yeast	Univ of Exeter & San Sebastian	Whey	cheese production	*Kluyveromyces marxianus*	3 l	research	Animal fodder	Willetts and Ugalde, 1987
Fungi	Univ of Messina	Orange peel	citrus processing	*G. Candidum & T. viride*	16 l	research	Animal fodder	Vaccarino *et al.*, 1989
Fungi	Univ of Botnova & Ankara	Citrus waste	citrus processing	*S. pulverul & A. niger*	250 ml	research	Animal fodder	Subasakan and Yasto, 1986
Yeast	Indian Reg Res Lab	Cassava	starch industry	*many different yeasts*	5 l	research	Animal fodder	Jamuna and Ramakrishna, 1989
Fu & Yeast	Buenos Aires Univ	Sugar cane stillage	alcohol production	*Candida utilis & many fungi*	500 ml	research	Animal fodder	Nudel *et al.*, 1987
Fungi	Cornell Univ	Sugar beet waste	beet processing	*Aspergillus niger (main)*	500 ml	research	Animal fodder	Hang, 1976
Yeast	Swedish Sugar Corp	Starch	maize or potato process	*E. Fibuligera & C. utilis*	10000 t/a	comm	Animal fodder	Solomons, 1983
Fu &Yeast	Cornell Univ	Sauerkraut brine	sauerkraut manufact	*C. Utilis & A. niger*	500 t/a	research	Animal fodder	Hang *et al.*, 1976
Fungi	Waterloo - Univ of Waterloo	P & P: solid waste	prim clarif sludge	*Chaetomium cellulolyticum*	2 l	research	Animal fodder	Paument et al., 1979 Moo-Young *et al.*, 1979
Fungi	Univ of Bophal	P & P: effluent	bagasse based effl	*Schizophyllum commune*	500 ml	research	Colour removal	Behare & Prasad, 1988
Fungi	Swedish Forest Prod Res Lab	P & P: effluent	'white' water	*Sporotrichum pulverulentum*	14 l	research	Animal fodder	Ek & Eriksson, 1980
Yeast	Waldhof - Boise-Cascade	P & P: SSL	stripped SSL	*Candida utilis*	5000 t/a	comm	Animal fodder	Solomons, 1983
Yeast	Waldhof - Cellul. Attisholz AG	P & P: SSL	stripped SSL	*Candida utilis*	10000 t/a	comm	Animal fodder	Solomons, 1983
Yeast	Sandwell International Inc	P & P: SSL	hardwood pulp	*Candida utilis*	t/a	proposal	Snack food	Andersen, 1979
Fungi	Pekilo SCP	P & P: SSL	acid bisulphite pulp	*Paecilomyces varioti*	10000 t/a	comm	Animal fodder	Romantschuk & Lehtomäki, 1978
Fungi	Univ of Pretoria	P & P: SSL	bisulphite pulp	*Geotrichum candidum*	13.5 l	research	Animal fodder	Kilian & Pretorius, 1989
Fungi	Univ of Quebec	P & P: SSL	Na-bisulphite pulp	*mixed culture*	400 l	research	Animal fodder	Lo *et al.*, 1978
Fungi	Mass. IT	P & P: SSL	acid bisulphite pulp	*Candida utilis*	5 l	research	Food yeast	Gpld *et al.*, 1981
Yeast	Univ of Natal	P & P: SSL	dissolving pulp	*Candida utilis*	2.5 l	research	Food yeast	McKee & Quicke, 1977
Yeast	C.G. Smith Chemicals	Molasses and stillage	Sugar cane waste	*Candida utilis*	1400 t/a	comm	Food yeast	Du Preez, 1990

APPENDIX B

MONOD KINETICS

(TO DETERMINE GROWTH PARAMETERS)

APPENDIX B

THE GROWTH KINETIC MODEL AND TECHNIQUE FOR ASSESSING GROWTH KINETIC PARAMETERS OF *ASPERGILLUS FUMIGATUS*

1. THE GROWTH KINETIC MODEL

- The growth kinetic model is based on an empirical equation first proposed by Monod (1949), which expresses the relationship between the specific growth rate constant and substrate concentration:

$$\mu = \frac{\mu_m . S}{K_s + S} \qquad \text{.....(B-1)}$$

By re-writing the above equation it can be seen that substrate concentration inside a reactor can be controlled by controlling the specific growth rate of the organism:

$$S = \frac{\mu . K_s}{\mu_m - \mu} \qquad \text{.....(B-2)}$$

To show how the growth kinetic model is related to operational and control parameters in describing the performance of a bioreactor system, the model can be substituted into mass balance equations for X and S. This will be done for a continuous-flow, completely mixed reactor system such as the single CSTR without recycle, whise is depicted in Figure B-1.

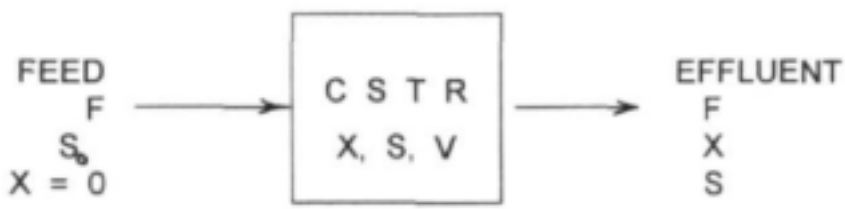

Figure B-1: CSTR without recycle - schematically

The mass balance for X is given as follows:

Mass rate of change in X	=	rate of change due to growth	+	rate of change due to decay	+	rate of change due to outflow

or:

$$V\frac{dX}{dt} = \mu.X.V - V.b.X - F.X \qquad ..(B\text{-}3)$$

or, by substituting for μ from the Monod model (Equation B-1), Equation B-4 is obtained:

$$V\frac{dX}{dt} = \left(\frac{\mu_m.S}{K_s+S}\right).X.V - V.b.X - F.X \qquad ....(B\text{-}4)$$

At steady state (dX/dt = 0) the above equation simplifies to:

$$\frac{F}{V} = \mu - b, \qquad \text{or} \qquad \mu = \frac{1}{\tau_h} + b \qquad ....(B\text{-}5)$$

By substituting μ from Equation B-5 in Equation B-2, S can be predicted from growth kinetic constants:

$$S = \frac{K_s\left(\frac{1}{\tau_h} + b\right)}{\mu_m - \left(\frac{1}{\tau_h} + b\right)} \qquad .....(B\text{-}6)$$

Thus, for a single CSTR, μ (and S) is only controlled by the mean hydraulic retention time, τ_h.

The equation that relates biomass formed due to substrate removed can be

derived similarly from a mass balance for substrate:

Mass rate of change in S	=	change due to growth	+	change due to inflow	+	change due to outflow

$$V\frac{dS}{dt} = -V\frac{\mu.X}{Y_g} + F.S_o - F.S \qquad \text{(B-7)}$$

By rewriting the above equation and substituting Equation B-5 for μ, the following equation for X is obtained:

$$X = \frac{Y_g(S_o - S)}{(1 + b^{\tau}h} \qquad \text{.....(B-8)}$$

From Equation B-8 it can be seen that the cell concentration in a CSTR depends only upon the hydraulic retention time in the reactor and the amount of substrate removed.

Similar mass balance Equation can be drawn up to arrive at the Equations summarised in Table 8.