Substrate utilisation by yeast in a spent sulphite liquor permeate

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Abstract

Spent sulphite liquor (SSL) is a waste effluent of the pulp industry. The refractory nature and high chemical oxygen demand (COD) of the effluent creates a serious pollution problem that is difficult and expensive to dispose of satisfactorily. Ultrafiltration of SSL (to recover chemicals), produced a permeate containing mainly D-xylose (22,5 g.ℓ$^{-1}$) and acetic acid (10,1 g.ℓ$^{-1}$). *Candida utilis* was most effective amongst a number of yeasts in the utilisation of these substrates and its growth resulted in a 57% reduction of the COD. The inhibition of *C. utilis* growth by sulphite and acetic acid was alleviated by steam treatment and pH control of SSL. Yeast protein could be recovered from the SSL as a valuable by-product and may reduce the costs of final treatment of SSL before discharge into the environment.

Introduction

Spent sulphite liquor (SSL) is a waste product of the pulp industry during a process by which wood chips are delignified by cooking with acid bisulphite. The insoluble cellulose fraction produced by the process is used to manufacture various products. SSL contains dissolved solids such as lignosulphonates and hemicellulose hydrolysis products. The relative amounts of these products are dependent upon the source of the wood (Hajny, 1981; Mueller and Walden, 1970). Consequently the effluent has a high COD and is a disposal problem. To alleviate the pollution problem, SSL has served as a substrate to produce yeast protein (Goldberg, 1985; Holderby and Moggio, 1960; Inskeep *et al.*, 1951; Rimmington, 1985; Walker and Morgen, 1946) although the use of a permeate has not been previously investigated.

Only a small proportion of the organic load (20%) is readily metabolised by yeast such as *C. utilis* (Camhi and Rogers, 1976) since up to 70% is lignosulphonate which is recalcitrant to microbial utilisation (Kosaric *et al.*, 1981). Separation of lignosulphonates from other organic molecules would yield a liquor more amenable to yeast growth, while the lignin fraction may be used as a source of valuable chemicals. The purpose of this study was to evaluate the ability of yeast to utilise sugars and acetic acid present in a permeate produced by ultrafiltration (Thomas, 1985), so that a protein by-product may be recovered while simultaneously reducing the pollution load of the effluent.

Materials and methods

Organisms

Yeasts were obtained from the departmental culture collection or were isolated from the soil surrounding a pulp mill at Umkomaas, Natal.

Growth media

The yeasts were cultivated in supplemented SSL permeate or a basal medium with various sources. The SSL was the effluent produced at a pulp mill (Umkomaas, Natal) when wattle (*Acacia mearnsii*) or eucalyptus (*Eucalyptus grandis*) wood chips are cooked with acidified calcium sulphite. SSL was ultrafiltered in order to obtain a clear permeate consisting mainly of D-xylose and acetic acid as carbon sources (Table 1). The concentrations of the components varied slightly between batches. Ultrafiltration was conducted by diffusing SSL at 55°C under pressure of 1,9 mPa through a tubular membrane with an 8 000 molecular mass cut-off at a rate of 23 ℓ.min^{-1} and flux of 13 ℓ m^{-2}.h^{-1}. This treatment reduced the COD of the SSL from 220 g.ℓ$^{-1}$ to 5,9 g.ℓ$^{-1}$. In some instances the sulphite concentration of the SSL permeate was reduced by bubbling steam at 94°C through the permeate for 30 min. The sulphite concentration decreased exponentially until approximately 20 per cent of the original amount remained (Fig. 1). The pH was adjusted to 6 with 10N NaOH and KH_2PO_4 (4 g.ℓ$^{-1}$) was added to the permeate. A precipitate formed and was removed by filtration (Whatman No.1). Each litre of permeate was supplemented with a concentrated basal medium (50 mℓ), concentrated trace salts (10 mℓ) and 1 g yeast extract (YE) (Difco). The filter-sterilised basal medium concentrate consisted of (per litre):$(NH_4)_2SO_4$, 80 g; $MgSO_4.7H_20$, 8 g; KH_2PO_4, 160 g and NaCl, 1,6 g; pH 6. The filter-sterilised trace salts concentrate consisted of: $CaCl_2.2H_20$, 2 g; $MnSO_4.4H_20$, 1 g; $FeSO_4.7H_20$, 0,5 g and 0,1N HCl, 1ℓ.

The synthetic medium consisted of 50 mℓ basal medium concentrate, 10 mℓ trace salts concentrate and YE (1 g.ℓ$^{-1}$) was made up to a litre with sterilised H_2O. D-xylose, D-glucose, sodium acetate were added in the concentrations indicated in the text. The carbon sources and YE were added to the synthetic medium as filter-sterilised concentrates. The pH was re-adjusted to 6.

Inoculum preparation

The inoculum was prepared by transferring a loopful of the stock culture to 25 mℓ synthetic medium in a 250 mℓ Erlenmeyer flask containing D-glucose (10 g.ℓ$^{-1}$), trace salts concentrate (1 mℓ) and YE (1 g.ℓ$^{-1}$). The culture was grown for 12 h at 30°C on an orbital shaker (180 r. min^{-1}; 27,5 mm throw). The cells were harvested by centrifugation (7 000 g for 15 min) and washed twice by resuspension in sterile H_2O. The washed cells were resuspended in 25 mℓ sterile H_2O and used to inoculate the growth medium.

Shake flask experiments

Supplemented SSL permeate (106 mℓ) in 500 mℓ Erlenmeyer flasks equipped with side-arm cuvettes was inoculated with 2 mℓ washed yeast culture and subjected to vigorous shaking (180 r. min^{-1}; 27,5 mm throw) at 30°C until cell growth reached stationary phase. The culture absorbance was monitored with a

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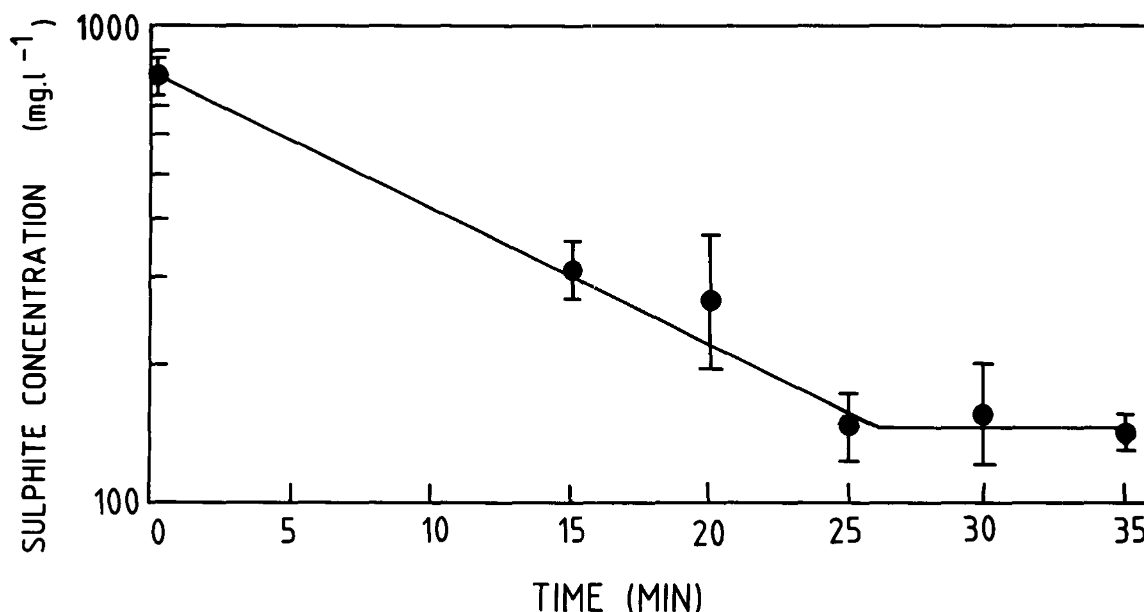

Figure 1
Removal of sulphite by steam treatment at 94°C. The line between 0 and 25 min was fitted by regression analysis (r = – 0,948; n = 12). The bars represent the standard deviation of the means.

Klett-Summerson clorimeter at 640 nm. At suitable intervals a 10 mℓ sample was withdrawn, centrifuged at 7 000 g for 15 min and the supernatant fluid and cell pellet were retained for analysis.

For the experiments conducted in the synthetic medium, D-xylose, sodium acetate or D-xylose/sodium acetate mixtures were added as indicated in the text. The medium (106 mℓ) was inoculated with 2 mℓ washed yeast culture and treated as described for the permeate.

Fermentation experiments

SSL (1 060 mℓ) supplemented as indicated above with concentrated basal medium, concentrated trace salts and YE in a 2,5 ℓ reactor (Multigen F 2000; New Brunswick Scientific Co., Edison, NJ, USA) was inoculated with 25 mℓ washed yeast culture. The pH was maintained at 6 with a pH-stat (T and C Scientific, Model 1003) using 5N HCl and 5N NaOH. The stirring speed was set at 700 r. min^{-1}, the air flow rate at 1 ℓ.min^{-1} and the temperature at 30°C. At suitable intervals, 20 mℓ was withdrawn from the reactor, centrifuged and the cell pellet and supernatant fluid were retained for analysis.

Analytical techniques

The dry biomass was determined gravimetrically by drying washed cell pellets to constant mass at 105°C. Sugar and acetic acid concentrations were determined by high performance liquid chromatography (Watson *et al.*, 1984) and gas chromatography (Du Preez and Lategan, 1978) respectively. The COD and sulphite concentration were determined as described by Taras *et al.* (1976). Yeast cells were enumerated with a haemacytometer.

The biomass yield coefficient ($Y_{x/s}$) was calculated from the equation

$$Y_{x/s} = \frac{X - Xo}{S_o - S}$$

where X and Xo are the final and initial biomass concentrations (grams per litre) respectively while So and S are the initial and final total substrate (xylose, acetic acid, glucose) concentrations (grams per litre).

The volumetric productivity (P) was calculated from the equation

$$P = \frac{X - Xo}{t}$$

where t is the cultivation time.

Results

Most yeasts completely utilised glucose present in SSL permeate during the 72 h incubation period (Table 2). The ability to utilise xylose differed between yeast strains. All yeasts were able to utilise acetic acid. However, substrate utilisation by yeast, especially *Pichia stipitis*, was poorer in untreated SSL permeate than steam-treated SSL permeate. This could be ascribed to the presence of a greater amount of sulphite in untreated SSL permeate. Steam treatment reduced the sulphite concentration although other volatile toxic components such as furfural might also be removed (Camhi and Rogers, 1976). The final yeast count in the stationary growth phase showed that the *C. utilis* strains gave the highest yeast count in untreated SSL permeate whereas in the steam-treated SSL permeate all strains except the unknown isolates gave similar cell numbers. Based on yeast count and the utilisation of glucose, xylose and acetic acid in the untreated SSL permeate, *C. utilis* ATCC 9256 was chosen for further study.

Production of protein from SSL permeate by *C. utilis* is dependent upon efficient conversion of xylose and acetic acid in SSL permeate to biomass. When *C. utilis* ATCC 9256 was grown in basal medium, D-xylose (20 g.$ℓ^{-1}$) and acetic acid (10 g.$ℓ^{-1}$) were rapidly used (Figs. 2A and B). When a mixture of D-xylose

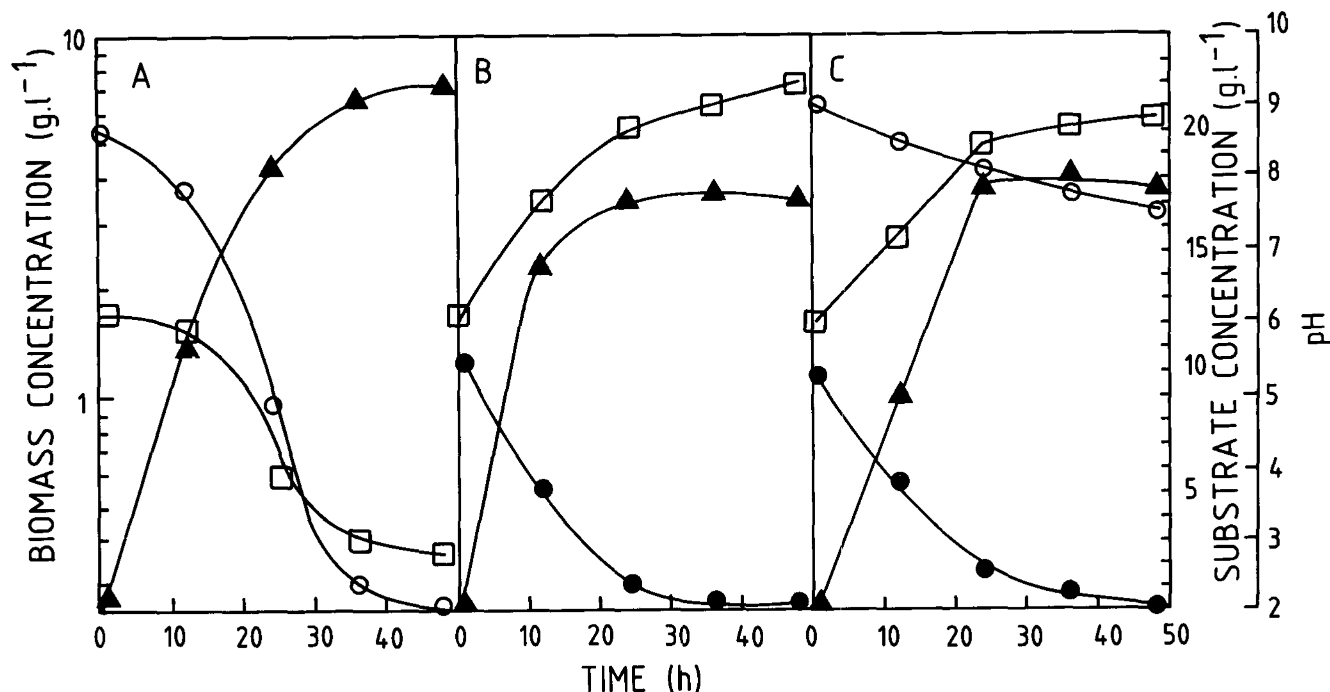

Figure 2
Growth in shake flasks of ***Candida utilis*** *ATCC 9256 in basal medium containing yeast extract (1 g.ℓ$^{-1}$) with 20 g.ℓ$^{-1}$ D-xylose (A), 10 g.ℓ$^{-1}$ acetic acid (B) and 20 g.ℓ$^{-1}$ D-xylose and 10 g.ℓ$^{-1}$ acetic acid (C) as main carbon sources. Biomass concentration (▲); D-xylose (○); acetic acid (●) and pH (□).*

(20 g.ℓ$^{-1}$) and acetic acid (10 g.ℓ$^{-1}$) was present in the medium, the acetic acid was completely used but not the D-xylose (Fig. 2C). Growth on acetic acid resulted in an increase in pH which may inhibit xylose utilisation.

The kinetics of growth by *C. utilis* ATCC 9256 on various concentrations of D-xylose, acetic acid and D-xylose/acetic acid mixtures are shown in Fig. 3. The specific growth rate was largely unaffected by increasing acetic acid concentrations. However, the biomass concentration showed little increase when the acetic acid concentration was increased from 5 to 15 g.ℓ$^{-1}$ and the yield coefficient decreased accordingly from 0,53 to 0,30. This may be related to the toxicity of acetic acid (Sestakova, 1979) or the pH increase from acetic acid utilisation (Chang, 1985). The pH dependence of acetic acid toxicity to *C. utilis* is shown by a decrease in yield coefficient from 0,38 to 0,27 when *C. utilis* was grown on 10 g.ℓ$^{-1}$ acetic acid at pH 6,0 and 5,3 respectively (data not shown). The specific growth rate and biomass yield showed little change with increasing D-xylose concentration while the biomass concentration increased. These parameters were lower than those observed with 5 g.ℓ$^{-1}$ acetic acid, but similar to those obtained with higher concentrations of acetic acid. In most instances mixtures of D-xylose and acetic acid gave kinetic values intermediate between those observed on D-xylose and acetic acid alone.

When *C. utilis* ATCC 9256 was grown in SSL permeate in a fermenter with pH held at 6, acetic acid and D-xylose were simultaneously consumed (Fig. 4A) without the inhibition of D-xylose consumption as observed in shake flasks (Fig. 2C). The pattern of acetic acid and D-xylose utilisation in untreated SSL permeate was similar to that observed in steam-treated SSL permeate but slower (Fig. 4B). Steam treatment of SSL permeate allowed *C. utilis* ATCC 9256 to grow more rapidly and produced biomass yields (Table 3) similar to those observed in shake flasks with synthetic medium containing similar concentrations of acetic acid and D-xylose (Fig. 3). Steam treatment of SSL permeate also resulted in a greater biomass concentration and volumetric productivity although it did not affect the final COD removal (Table 3.)

TABLE 1
TYPICAL COMPOSITION OF SPENT SULPHITE LIQUOR PERMEATE FROM PULP MILL AT UMKOMAAS

Component	Concentration
D-Glucose	1,3 g.ℓ$^{-1}$
D-Xylose	22,5 g.ℓ$^{-1}$
L-Arabinose	0,5 g.ℓ$^{-1}$
Acetic acid	10,1 g.ℓ$^{-1}$
Sulphite (as Na_2SO_3)	0,8 g.ℓ$^{-1}$
COD	54,9 g.ℓ$^{-1}$
pH	1,9

TABLE 2
GROWTH OF YEAST ON SPENT SULPHITE LIQUOR PERMEATE AT 30°C FOR 72 H

Culture	Cell count ($x10^8.m\ell^{-1}$)	Components utilised ($g.\ell^{-1}$) Glucose	Xylose	Acetic acid
SSL permeate (untreated)[a]				
Yeast isolate 1	1,6	2,3[c]	4,3	8,5
Yeast isolate 2	1,2	2,3[c]	4,6	7,6
Candida utilis CSIR-Y12	2,3	2,3[c]	1,2	8,7
Candida utilis CBS 890	2,8	2,3[c]	19,8[c]	3,9
Candida utilis ATCC 9256	3,1	2,3[c]	16,9	7,4
Candida steatolytica CSIR-Y535	2,1	1,8	1,1	8,3
Pichia stipitis CSIR-Y633	0,3	0,1	0,2	1,0
SSL permeate (steam-treated)[b]				
Yeast isolate 1	1,0	2,0[c]	5,4	7,9
Yeast isolate 2	1,5	2,0[c]	6,0	8,0
Candida utilis CSIR-Y12	3,2	2,0[c]	17,2[c]	8,0
Candida utilis CBS 890	2,9	2,0[c]	17,2[c]	6,4
Candida utilis ATCC 9256	3,1	2,0[c]	17,2[c]	7,4
Candida steatolytica CSIR-Y535	3,9	2,0[c]	17,2[c]	7,0
Pichia stipitis CSIR-Y633	2,9	2,0[c]	17,2[c]	7,5

[a] The permeate consisted of: D-glucose, 2,3 $g.\ell^{-1}$; D-xylose, 19,8 $g.\ell^{-1}$; acetic acid, 8,9 $g.\ell^{-1}$ and sulphite, 0,8 $g.\ell^{-1}$
[b] The permeate consisted of: D-glucose 2,0 $g.\ell^{-1}$; D-xylose, 17,2 $g.\ell^{-1}$; acetic acid, 8,2 $g.\ell^{-1}$ and sulphite, 0,16 $g.\ell^{-1}$. The sulphite concentration was reduced by steam-treatment for 30 min at 94°C.
[c] Complete substrate utilisation.

TABLE 3
KINETIC PARAMETERS OF *CANDIDA UTILIS ATCC 9256* GROWTH IN UNTREATED AND STEAM-TREATED SSL PERMEATE IN A FERMENTER AT pH 6 AND 30°C

Parameter	Steam-treated SSL permeate	Untreated SSL permeate
Specific growth rate (h^{-1})	0,30	0,13
Biomass ($g.\ell^{-1}$)	9,9	8,3
Yield (g biomass. total substrate utilised^{-1})	0,38	0,34
Volumetric productivity $g.\ell^{-1}.h^{-1}$	0,41	0,22
COD removed (%)	57	57
Final COD ($g.\ell^{-1}$)	23,6	23,6

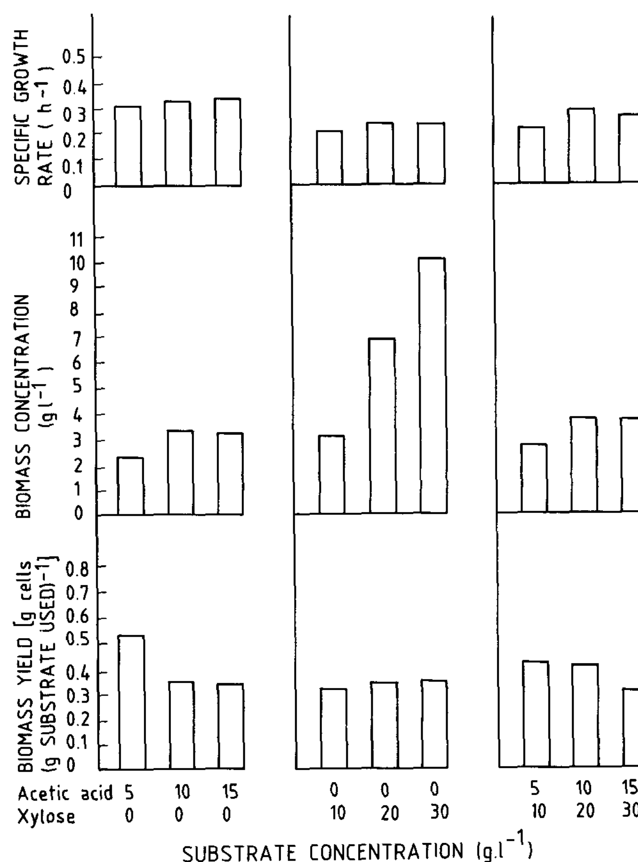

Figure 3
Effect of substrate concentration on specific growth rate, biomass concentration and substrate yield of ***Candida utilis*** *ATCC 9256 when grown at 30°C.*

Discussion

C. utilis was the most effective yeast in the utilisation of the principle components of the SSL permeate. This organism is commonly used in single cell protein (SCP) production because of its ability to utilise hexoses, pentoses and organic acids as found in the permeate and is widely accepted for feed and food purposes (Goldberg, 1985; Litchfield, 1983). The SSL permeate contains various components such as sulphite and acetic acid that inhibit yeast growth (Table 1) and the efficient conversion of the substrate to yeast biomass is dependent upon the elimination of these toxic factors. Steam treatment partially reduced the sulphite concentration and allowed more rapid growth (Tables 2 and 3). Acetic acid, however, cannot be easily removed and furthermore can serve as a substrate for yeast growth. At the optimum pH for *C. utilis* growth on carbohydrates (pH 4,5) acetic acid is especially toxic and its toxicity is concentration dependent

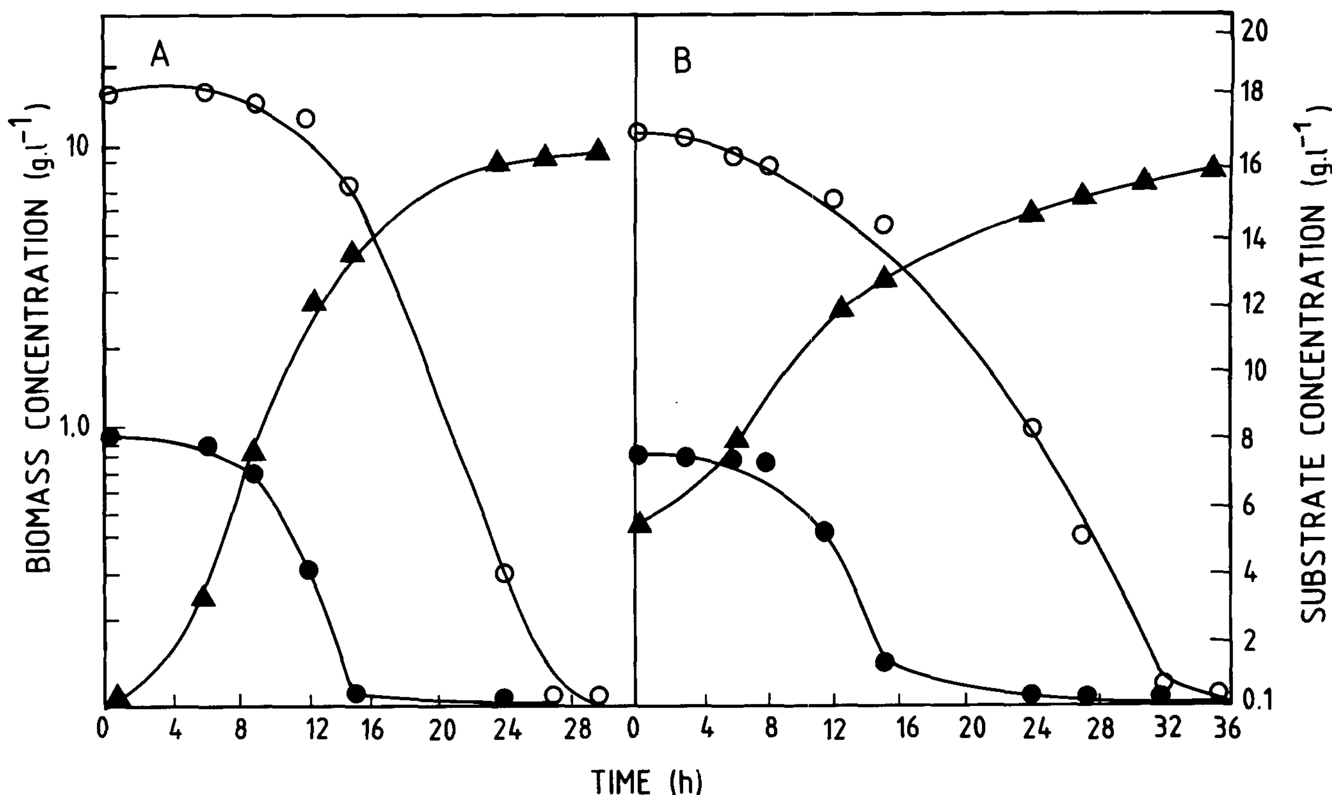

Figure 4
Growth of ***Candida utilis*** *ATCC 9256 in steam-treated (A) (0,35 g.ℓ$^{-1}$ sulphite) and untreated (B) (1,15 g.ℓ$^{-1}$ sulphite) SSL permeate in a fermenter at 30°C. D-xylose (○); acetic acid (●), biomass concentration (▲).*

(Chang, 1985; Sestakova, 1979). Although most plants producing *C. utilis* operate at pH 4,5 (Holderby and Moggio,1960; Gold *et al.*,1981), acetic acid concentration in the SSL permeate requires that the fermentation be conducted at pH 6 in order to minimise the toxic effects of the undissociated acetic acid. In acetic acid D-xylose mixtures, acetic acid was preferentially utilised. The resultant pH increase inhibited D-xylose utilisation (Fig. 2) but could be circumvented by controlling the pH at 6 (Fig. 4).

The biomass yields observed in this study (Table 3) were lower than those reported by other workers growing *C. utilis* on SSL (Camhi and Rogers, 1976; Gold *et al.*, 1981; Litchfield, 1983). This may be ascribed to the high acetic acid concentration. Hueting and Tempest (1977) found that the biomass yield of *C. utilis* decreased when the acetic acid concentraion increased and the acetic acid was oxidised rather than assimilated. Growth of *C. utilis* in an acetic acid-limited continuous culture could reduce this effect.

Between 3 and 4,6 x 10^5 m^3 SSL per year is discharged at Umkomaas, Natal (McKee and Quicke, 1977; Thomas, 1985). Based on the kinetic data reported here (Table 3), a steam-treated SSL permeate will yield between 2 970 and 4 554 tons yeast biomass per year. Plants in the USA currently produce similar amounts of SCP from SSL (Litchfield, 1983; Pearl, 1982). This product could be sold as an animal feed in competition with cotton seed cake and soya bean meal that currently cost between R970 and R1 220 per ton on the local market. Therefore a gross income of between R2,8 and R5,5 million per year could be recovered from the sale of the yeast protein produced from the effluent discharge at Umkomaas. The costs of raw materials for SCP production from sugar substrates typically range between 40 and 50 per cent of the total production costs (Moo-Young, 1977; Litchfield, 1983). The use of SSL permeate as main substrate could reduce the production cost considerably. However, a detailed analysis is necessary in order to determine the economic feasibility of yeast protein production from SSL permeate. The economics of SCP production from SSL may be improved by increasing the cell density in the fermenter and by greater productivity (Moo-Young, 1977). Concentration of SSL (by evaporation) or addition of another substrate (Rychtera, 1978) would increase cell density in the fermenter and thus lower harvesting costs. However, increased substrate preparation costs could outweigh any savings.

The volumetric productivity of yeast growth is an important factor in the viability of SCP production. Our data show a fourfold increase in productivity compared to similar previous batch culture studies on the same effluent (McKee and Quicke, 1977) and could be due to the pH (4 to 4,5) used in their studies. Our productivity values were similar to other batch culture studies on SSL (Gold and Mohagheghi, 1978). Continuous culture is used in most instances to grow yeast on SSL since productivity can be significantly increased (Prior, 1984). A further 2,5-fold (from 2,7 to 6,1 g.ℓ.$^{-1}$h^{-1}) increase in productivity was observed by coupling cell recycle to continuous culture (Gold *et al.*, 1981). Furthermore the cell concentration in the reactor increased from 9,5 to 24 g.ℓ$^{-1}$.

Yeast growth in SSL reduces the organic load of the effluent and when expressed in terms of biochemical oxygen demand, reductions of up to 80% have been reported (Camhi and Rogers,

1976; Holderby and Moggio, 1960; Hajny, 1981). We observed a 57% reduction in the COD of the SSL permeate (Table 3). However, a 89% COD reduction was obtained when the COD of the SSL before ultrafiltration (220 $g.\ell^{-1}$) was considered. Yeast production therefore offers a means of significantly reducing the COD of SSL but additional treatment would be necessary for total reduction of the remaining COD (23,6 $g.\ell^{-1}$). It does however offer a means of alleviating the costs of pollution abatement.

Acknowledgements

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