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Growth of Schizosaccharomyces *sp. on Skim Latex Serum*

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The aerobic growth of Schizosaccharomyces *sp on skim latex serum was studied The maximum yeast concentration of about 7 g/litre serum was reached after 30 h of cultivation on sulphuric acid coagulated serum and after 48 h on formic acid coagulated serum The average specific growth rates for the yeast grown on formic and sulphuric acid coagulated sera were 0 027 h^{-1} and 0 056 h^{-1} respectively Irrespective of the type of skim latex serum used the chemical oxygen demand reduction after four days of batch cultivation was about 80% indicating yeast cultivation can be used to treat rubber effluent*

Skim latex serum is derived from the coagulation of skim latex, a by-product of latex concentrate production The serum, containing a rich source of nitrogen, carbohydrates, proteins, lipids and trace metals[1], is the most polluting source in a latex concentrate factory Usually combined with liquid wastes from other sources in the latex concentrate factory, the serum is conventionally treated using an oxidation ditch or waste stabilisation ponds[2,3] These treatment systems are either costly or technically unsatisfactory[4]

Utilisation of the rubber serum to yield valuable substances can reduce the cost of effluent treatment or even generate additional revenue for rubber factories Recently, a process of concentrating and recovering the non-rubber solids present in the serum was developed[5] The concentrated serum has been used as a fertiliser, but can also be used as a substrate for the cultivation of various micro-organisms and in deriving pharmaceutical-grade chemicals

The process of concentrating the serum and converting it into a powder is energy intensive If the serum can be directly utilised without the need to concentrate and or convert it into a powder, the processing cost can be lower A promising approach to the direct utilisation of skim latex serum is in the production of yeast Yeast has a high protein content (about 52%) and has been used as an animal feed supplement[6]

Some yeasts, namely *Candida Saccharomyces, Hansula* and *Rhodotorula* have grown profusely in block rubber serum[7] In a study with *Candida utilis* using an optimum pH of 5 5 for growth and an incubation period of 24 h, about 5 – 8 g biomass litre serum was obtained[8] However an in-depth study on the growth of yeast on skim latex serum is still lacking

In this study, a yeast was isolated from a laboratory-scale anaerobic reactor and was tentatively identified as *Schizosaccharomyces* sp based on its morphological appearance and its manner of vegetative reproduction by fission This paper reports on the growth of this yeast on skim latex serum

MATERIALS AND METHODS

Isolation Procedure

About 10 ml of liquor from a laboratory-scale upflow anaerobic polyurethane filter treating skim latex serum was inoculated into a 100 ml sterile yeast-malt extract broth (Y-M broth 3 g yeast extract, 3 g malt

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extract, 5 g peptone and 10 g glucose in 1 litre distilled water, as first formulated by Haynes *et al*[9]). The pH of the broth was adjusted with 1 *N* HCl to 3.7 – 3.8 to encourage growth of yeast but not bacteria. The microbial culture grown on the Y-M broth was incubated in a 500 ml Erlenmeyer flask placed on a reciprocal shaker for 24 h at a temperature of 29°C – 30°C. After 24 h of incubation on the shaker, the yeast grown on the Y-M broth was isolated for a single yeast colony by a pour plating method using a Y-M agar medium at pH 5. A series of transfers of single yeast colonies by streaking on Y-M agar plates was carried out to obtain a pure single yeast colony. A yeast was isolated and tentatively identified as *Schizosaccharomyces* sp. based on its morphological appearance and its manner of vegetative reproduction by fission. The purified *Schizosaccharomyces* sp. was maintained on a Y-M agar slant and subsequently used in this study.

Preparation of Inoculum

For the first run, a loop of *Schizosaccharomyces* sp. maintained on the Y-M agar slant was transferred to a fresh Y-M agar slant and incubated at 30°C for 24 h. After the incubation, the yeast growing on the agar slant was aseptically scrapped and suspended in a 5 ml sterile Y-M broth. This suspension of yeast culture was added to a 500 ml sterile Y-M broth in a 2-litre fermentor (B. Braun BIOLAB) and incubated for about seven days under aerobic conditions at 26°C – 29°C. About 100 ml of this culture was used as an inoculum in the first run using skim serum as the growth medium. For the subsequent runs, the inoculum was not prepared from an agar slant as described but instead the inoculum for each subsequent run contained centrifuged yeast cells from the preceding run.

Culture Medium

The serum from the coagulation of skim latex with 10% (w/w) formic acid or 10% (w/w) sulphuric acid was used as the medium. Prior to the growth study, pH of the serum was adjusted with 2.5 *N* NaOH to 4.9 – 5.0 and the serum was autoclaved. About 900 ml of the serum after the solids had settled was used.

Growth Study

The yeast was grown on skim latex serum batch-wise at 30 C for four days. The initial volume of the culture medium with inoculum in a 2-litre fermentor (B. Braun BIOLAB) was 1 litre. The yeast suspension was agitated at 250 r.p.m. and aerated with air at 15 – 18 cm^3/min. The pH of the suspension was maintained at about 5.0 by a pH controller using 5 *N* H_3PO_4 and 2.5 *N* NaOH. Culture medium samples were taken twice daily (about 9 a.m. and 3 p.m.) during the four days of incubation

Analytical Methods

Suspended solids, volatile suspended solids (VSS) and chemical oxygen demand (COD) were analysed according to the methods described in *the Manual of Laboratory Methods for Chemical Analysis of Rubber Effluent*[10]. Reducing sugar was estimated by the method described by Miller[11]

RESULTS AND DISCUSSION

Growth of *Schizosaccharomyces* sp. on Skim Latex Serum

Figure 1 shows the growth curves for aerobic growth of *Schizosaccharomyces* sp. on serum obtained from the coagulation of skim latex with formic acid (first four experimental runs). Lag phase (no appreciable growth at the beginning of the batch cultivation) was observed in the first run. There were probably three main factors influencing the length of lag phase in this run: the growth stage, size of inoculum and the changes in nutrient composition. The inoculum for *Run 1* was prepared using a synthetic medium. The transfer of the yeast culture grown in the synthetic medium to the skim latex serum resulted in a time interval with non-multiplicative growth while new

enzymes and cofactors for metabolising new nutrients in skim serum were synthesised in the cell. In *Run 2*, the lag phase was not observed but the exponential growth rate was at a slower pace compared to those in subsequent runs. The inoculum for *Run 2* contained yeast cells from *Run 1* and thus the culture had adapted to the skim serum but probably not to a full extent as indicated by the slower growth. The cultures in subsequent runs were fully adapted to the skim serum because the inoculum for each subsequent run contained yeast cells from the preceding run. In these runs (*Runs 3* and *4*), maximum growth was reached in a shorter time (less than 50 h) mainly attributed to the larger size of inoculum.

Figure 2 shows the growth curves for aerobic growth of *Schizosaccharomyces* sp. on skim latex serum for the last three runs where sulphuric acid was used to coagulate skim latex. The yeast cultures in these runs were fully adapted to the skim serum because inoculation was carried out using yeast cells from the preceding run.

For yeast grown on skim serum obtained from formic acid coagulation of skim latex *(Figure 1)*, the maximum yeast VSS concentration (>7 g/litre serum) was reached after 48 h of fermentation. For yeast grown on skim serum obtained from sulphuric acid coagulation of skim latex *(Figure 2)*, the maximum yeast VSS concentration was slightly lower (about 6.8 g/litre serum) but was reached in a shorter time (about 30 h). Lacking in sulphate could probably be the main reason for the lower rate of yeast growth for serum from formic acid coagulation. Yeast requires 0.01 – 0.24 g sulphur per 100 g cell dry weight[12].

Stoichiometric and Kinetic Data

Table 1 gives the stoichiometric and kinetic data for the aerobic growth of *Schizosaccharomyces* sp. on serum obtained from formic acid and sulphuric acid coagulation of skim latex. The specific growth rate, μ, for the yeast grown on formic acid coagulated serum varied from 0.02 h^{-1} to 0.033 h^{-1} but for the same yeast species grown on sulphuric acid coagulated serum, μ varied from 0.041 h^{-1} to 0.068 h^{-1}. As explained earlier, lacking in sulphate could be the main reason for the lower specific growth rate for yeast grown on formic acid coagulated serum.

The specific growth rate of *Schizosaccharomyces* sp. on skim latex serum was generally low due to low concentration of reducing sugar in the serum (1.7 – 3.2 g sugar/litre). Specific growth rate ranges for many organisms considered for single-cell protein production[6] were 0.11 h^{-1} to 0.7 h^{-1}. Although the yeast cultivation on skim latex serum appears to be uneconomical for commercial production of single-cell protein due to low growth rate, it may be a viable approach for the treatment of rubber effluent considering current treatment systems using aerators are costly.

The values of yield factor and net yeast production for the yeast grown on formic acid coagulated serum were generally lower than the respective values for the yeast grown on sulphuric acid coagulated serum *(Table 1)*. As explained earlier for the specific growth rate, lacking in sulphate could be the main reason for the above differences.

COD Reduction

Chemical oxygen demand is important in effluent treatment and it is a measure of the total quantity of oxygen required for oxidation of organics to carbon dioxide and water. *Table 2* gives the reduction of soluble COD during aerobic growth of yeast on skim latex serum.

The first-order COD reduction rate coefficient values for the growth of the yeast on formic acid coagulated serum were comparable to those for the growth of the yeast on sulphuric acid coagulated serum *(Table 2)*. Sulphate which was indicated earlier to be enhancing the growth of the yeast was not significant in increasing the rate of utilisation of organics by the yeast.

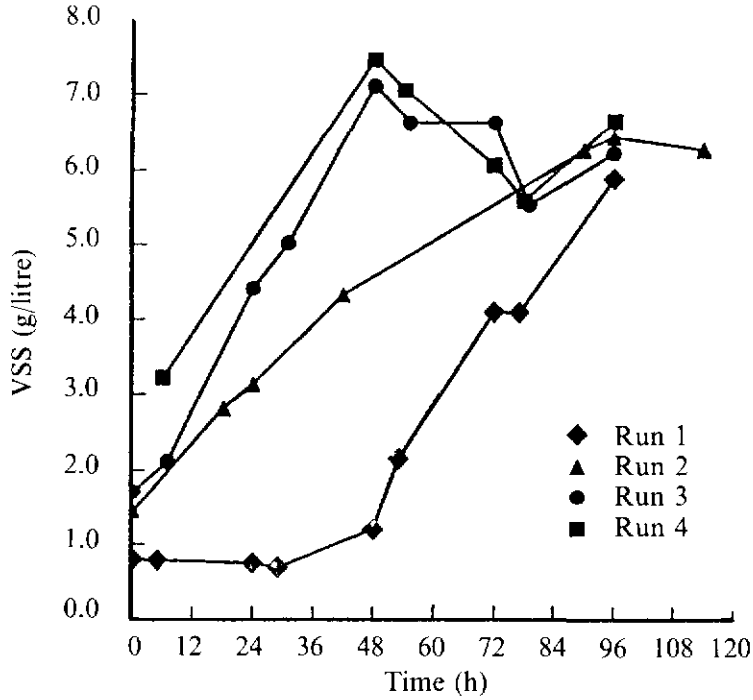

Figure 1. Aerobic growth of Schizosaccharomyces *sp. on serum from skim latex coagulation with formic acid.*

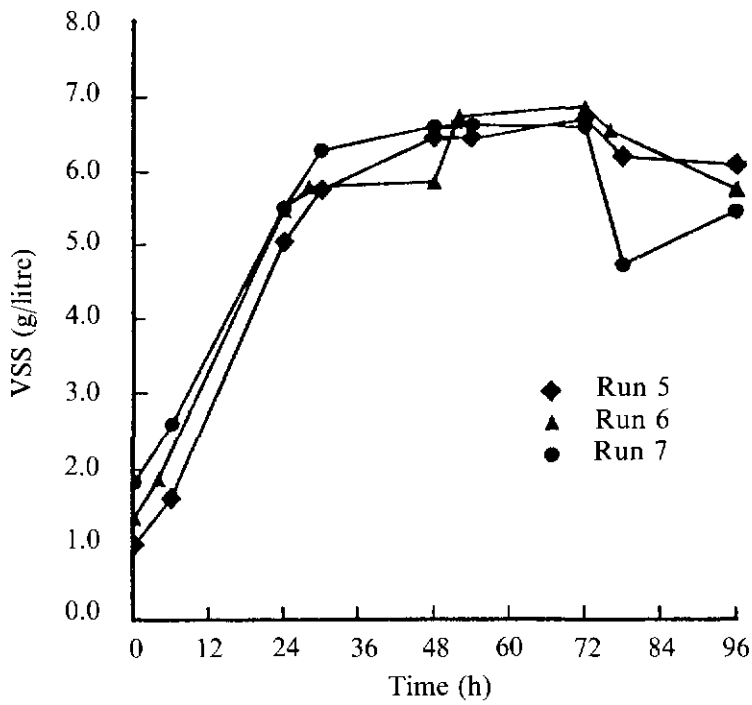

Figure 2. Aerobic growth of Schizosaccharomyces *sp. on serum from skim latex coagulation with sulphuric acid.*

TABLE 1 STOICHIOMETRIC AND KINETIC DATA FOR AEROBIC GROWTH OF *SCHIZOSACCHAROMYCES* sp ON SKIM LATEX SERUM

Run	Acid used in coagulating skim latex	Specific growth rate (h^{-1})	Yield factor (g VSS g COD)	Net yeast production (g VSS litre)
1	Formic	0 033	0 218	5 10
2	Formic	0 026	0 145	5 00
3	Formic	0 030	0 300	5 41
4	Formic	0 020	0 374	4 83
Average		0 027	0 259	5 09
5	Sulphuric	0 068	0 653	5 72
6	Sulphuric	0 058	0 493	5 52
7	Sulphuric	0 041	0 389	4 80
Average		0 056	0 512	5 35

TABLE 2 SOLUBLE COD REDUCTION DURING AEROBIC GROWTH OF *SCHIZOSACCHAROMYCES* sp ON SKIM LATEX SERUM

Run	Acid used in coagulating skim latex	First order COD reduction rate coefficient (h^{-1})	COD reduction after 4 days of batch cultivation (%)
1	Formic	0 033	82 4
2	Formic	0 015	74 9
3	Formic	0 029	80 7
4	Formic	0 027	83 8
Average		0 026	80 5
5	Sulphuric	0 019	82 0
6	Sulphuric	0 026	79 3
7	Sulphuric	0 029	82 4
Average		0 025	81 2

It was further observed that the COD reduction rate coefficient value of about 0 025 h^{-1} for the growth of the yeast on skim latex serum was comparable to that for the batch growth of *Candida obtusa* on liquid pineapple waste[13] (0 023 h^{-1})

Irrespective of the type of skim latex serum used, the COD reduction after four days of batch cultivation was about 80% *(Table 2)*, indicating yeast cultivation can be used to pre-treat rubber effluent A secondary effluent treatment system such as the high rate algal pond is still required to remove the remaining COD and nitrogen

CONCLUSION

A yeast tentatively identified as *Schizosaccharomyces* sp was found to effectively utilise skim latex serum for its growth

under aerobic conditions For the treatment of rubber effluent, yeast production from skim latex serum with over 80% reduction in chemical oxygen demand is technically feasible

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