Journal of Microbiology and Biotechnology
Vol. 5, No. 2, 114-116, 1995

Note

Yeast Biomass Production from Concentrated Sugar Cane Stillage Using a Thermotolerant *Candida rugosa*

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Concentrated Brazillian sugar cane stillage was used as a substrate for the yeast biomass production using *Candida rugosa* isolated from East Africa. At the optimum stillage concentration of 10% dry matter, biomass production was 20.4 g/l and COD reduction rate was 41%. The specific growth rate of the yeast was 0.17 h^{-1} and the corresponding productivity 0.91 g l^{-1} h^{-1} in the batch fermentation was observed at 40°C.

The increased demand for fuel alcohol causes environmental problems due to the increased production of stillage residues (9). For the better treatment we used stillages as the cultivation media for the feed yeasts production. For the economical production of yeast biomass, some East African strains showing thermo- and acid-tolerance were isolated through a screening program. As a result, *Candida rugosa* was selected to show high biomass yield and high specific growth rate in the stillage at 40°C (2). Kinetic behavior and flocculation characteristics of the strain at 20°C, 30°C and 40°C were also studied (8). Moreover, the growth of *C. rugosa*, the corresponding COD reduction and substrate assimilation in three different beet stillages were also reported (7). In the present study, concentrated sugar cane stillage from Brazil was investigated as a substrate for the biomass production of *C. rugosa*. The physicochemical characteristics of the stillage were also analyzed.

Microorganism

C. rugosa was isolated from Sudan in East Africa and identified by the method of Barnett (1). Stock culture of the strain was maintained on a stillage agar medium fortified with phosphorus.

Growth Medium

Concentrated sugar cane stillage was supplied by Center for Biotechnology and Chemistry, Foundation for Industrial Technology in Rorera, Brazil. The concentrate of 45% dry matter was diluted with distilled water to needed concentrations. After diluted to proper concentrations, the stillages were centrifuged to remove suspended matter.

Fermentation Conditions

As a substrate for starter culture, the stillage of 10% dry matter fortified with 0.5·g/l phosphorus was used. 0.2 ml of starter culture was added to 10 ml of sterilized stillage in 100 ml-Erlenmeyer flask and incubated in shaking incubator, which was controlled constantly at 170 shaking frequency per minute at 40°C for 48 hours. For the kinetic study, the batch fermentation was conducted in a 1 litre fermenter. The fermentation conditions were as described previously (7).

Analytical Methods

Biomass was measured by dry weight method after centrifugation (10 min at 5,000 g). COD of the stillages were measured by the standard method of DIN 38409 with a thermometer TR 105 (Merck, Darmstadt) (4). Protein concentration was determined by the Biuret method as described by Herbert et al. using bovine serum albumin as a standard (5) and crude protein by Kjeldahl method. Total acidity was measured by titration against 0.01 M NaOH using bromomethylblue as an indicator and expressed as lactic acid equivalents. Total carbohydrate was determined by the Anthrone method (11) while glycerol was determined by the enzymatic method of Boeringer Mannheim (3).

Characteristics, Composition, and Optimization of Nutritional Composition of Sugar Cane Stillage

Table 1 shows the sugar cane stillage containing high amount of insoluble suspended solid and ashes. In

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Key words: Yeast biomass production, sugar cane stillage treatment, COD reduction, *Candida rugosa*

addition, it contained low amount of reducing sugar despite its high content of carbohydrate. This result has a significance regarding the high fiber content in the turbid stillage. L-lactic acid content of the sugar cane stillage was higher than that of other stillages (10). Especially, the glycerol content (22.6 g/l) was higher than the total carbohydrate content. This explains that the yield of ethanol production from the sugar cane juice might be low (6).

In comparison with sugar beet stillages, the contents of Ca, Mg and ortho-phosphate in sugar cane stillage were much higher (Ca: 3~6 times, Mg: 17~57 times and o-phosphate: 6~17 times). The relativeness among COD, nitrogen and ortho-phosphate showed a distinct N-deficit and enough phosphate content as well as relatively high organic load in the stillage. The ratio of COD/organic dry matter of the the sugar cane stillage was 1.3 times that of the sugar beet stillage (2).

Table 1. Chemical and physical characteristics of sugar cane stillage

Component and Physical Characteristics	Test Results
COD, soluble	104.2 (g/l)
Dry Matter, soluble	107.4 (g/l)
Dry Matter, organic	79.0 (g/l)
Dry Matter, suspended	20.2 (g/l)
Nitrogen	2.23 (g/l)
Protein, Biuret	27.2 (g/l)
Carbohydrate	18.3 (g/l)
Reducing Sugar	4.5 (g/l)
D-Glucose	0.0 (g/l)
D-Fructose	0.0 (g/l)
Sucrose	0.0 (g/l)
Titrable Acid, as lactate	8.2 (g/l)
D-Lactic Acid	1.8 (g/l)
L-Lactic Acid	2.5 (g/l)
Glycerol	22.6 (g/l)
Ethanol	0.0 (g/l)
Ca	1.76 (g/l)
Mg	2.05 (g/l)
ortho-Phosphate	0.38 (g/l)
Ash	28.4 (g/l)
pH	4.0
Osmotic Pressure	22.9×10^5 Pa
Density	1.038
Electrical Conductivity	22.5 mS cm^{-1}
COD : N : ortho-Phosphate	46.73 : 1 : 0.17

Through the preliminary experiments of P, N, Mg, minerals and vitamin complex addition, only N-deficit was confirmed. The proper amount of N-addition was thought to be 1 g/l by the quantitative determination experiment (Table 2). 0.5 g/l of N-addition increased the biomass production almost twice and COD reduction 1.7 times of those of the non-added one, but the crude protein content of the harvested biomass was still remained in low level. By the addition of 1.0 g/l N, the crude protein content increased highly, while the COD reduction rate increased only slightly. The $Y_{X/COD}$ and $Y_{CPP/COD}$ reached their maximum at 2.0 g/l N-addition, in which the yeasts had the highest biosynthetic rate and the lowest maintenance energy consumption, and then decreased.

Table 3 shows the influence of substrate concentration of sugar cane stillage on the yeast biomass production with 1 g/l N-addition as urea. At over 12.5% dry matter of the medium, the metabolic capability of yeast was inhibited by the increased osmotic pressure and possibly by the lower oxygen concentration in the dense medium. On the other hand, the protein content of the yeast biomass produced increased continuously. Under the concentration of 10% dry matter of the stillage medium, biomass production was not high enough, but COD reduction rate was almost constant around 41%. The $Y_{X/COD}$ and $Y_{CPP/COD}$ were highest at 9 and 10% dry matter, respectively. As a conclusion, the optimum dry matter content of the sugar cane stillage medium was considered to be 10%.

Batch Fermentation Kinetics and Assimilation Procedure

Fig. 1 shows the assimilation procedure of *C. rugosa* on 8% dry matter of sugar cane stillage at 40°C. The specific growth rate of *C. rugosa* was 0.17 h^{-1} during active growth phase and the corresponding productivity of the yeast biomass was 0.91 $gl^{-1}h^{-1}$. Both values were somewhat lower than those in case of sugar beet stillages.

Table 2. Quantitative determination of N-addition in sugar cane stillage for the biomass production using *C. rugosa* by shaking culture at 4°C for 2 days

*N-Addition(g/l)	pH	X(g/l)	CPC(%)	CPP(g/l)	COD_{red} .(%)	$Y_{X/COD}$	$Y_{CPP/COD}$
5.0	6.5	19.20	29.0	5.57	39.9	0.46	0.13
4.0	6.5	20.38	29.2	5.95	40.9	0.48	0.13
3.0	6.5	20.00	29.3	5.86	38.1	0.50	0.15
2.0	6.5	20.86	29.3	6.11	37.7	0.53	0.16
1.0	6.5	20.24	29.0	5.87	37.2	0.52	0.15
0.5	6.4	19.42	25.1	4.87	36.6	0.51	0.13
0.0	5.9	10.5	20.0	2.10	21.5	0.47	0.09

*Nitrogen was added as urea, X: Biomass production, CPC: Crude Protein Content, CPP: Crude Protein Production, COD red.: COD reduction rate, $Y_{X/COD}$ =Biomass production/COD reduction(g/g), $Y_{CPP/COD}$=Crude protein production/COD reduction (g/g)

Table 3. Influence of medium concentration of sugar cane stillage for the biomass production and COD reduction using *C. rugosa* by shaking culture at 40°C for 2 days

Dry Matter(%)	pH	X(g/l)	CPC(%)	CPP(g/l)	$COD_{red.}$(%)	$Y_{X/COD}$	$Y_{CPP/COD}$
20.0	4.3	9.92	33.9	3.36	14.9	0.32	0.11
15.0	4.8	14.78	33.3	4.92	26.8	0.35	0.12
12.5	5.3	16.53	33.0	5.45	31.6	0.40	0.13
10.0	6.5	20.41	30.4	6.21	40.5	0.48	0.15
7.5	6.9	15.14	31.0	4.69	40.8	0.48	0.15
5.0	6.9	10.38	32.7	3.39	41.0	0.49	0.16
2.5	6.8	4.69	34.0	1.60	40.7	0.44	0.15

[a] for illustration of symbols, see Table 2

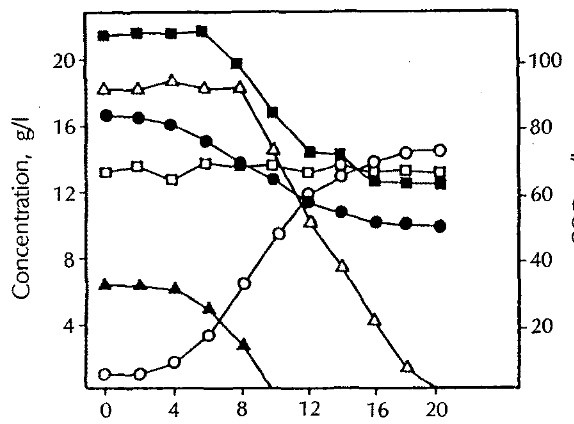

Fig. 1. Batch fermentation kinetics, COD reduction, and consumption of main assimilable compounds in sugar cane stillage during the growth of *C. rugosa* at 40°C.
○ Biomass, ● COD, ■ Protein, △ Glycerol, □ Carbohydrate, ▲ Titrable acid as lactate.

The COD reduction rate was 40%. In the beginning of the assimilation procedure, organic acids were consumed relatively rapidly. As was observed in sugar beet stillages, the protein content in the medium was constant in the first 6 hours, and thereafter the protein assimilation began (7). The protein assimilation rate reached 42% at the end of the fermentation. During the overall fermentation period, carbohydrate content was almost constant and no assimilation was observed even though its content was high in the stillage. As shown in analyses results (Table 1), sugar cane stillage did not contain D-glucose and D-fructose. Although sugar cane stillage contained high amount of glycerol, its assimilation could begin after 8 hours, and they were all consumed. The yeast *C. rugosa* grew in sugar cane stillage without any flock formation during fermentation period. After 12 hours holding after harvested, the yeast suspension formed flocks, however, still comprised unsedimentated yeasts, which were yellowish and much brighter than those grown in sugar beet stillage (7).

In spite of high assimilation rate of defined compound such as glycerol and organic acids, the COD reduction rate of the sugar cane stillage was 41%. This suggests that sugar cane stillage contained unassimilable compounds like fiber or unkown ones. The stillage lacked only in nitrogen, which can be covered by the addition of chief nitrogen source such as urea. Sugar cane stillage would be a good and cheap substrate for the feed yeast production if the energy consumption could be lowered in the scaled up processes.

REFERENCES

1. Baerwald, G. and S.H. Hamad. 1984. The presence of yeasts in Sudanes cane sugar factories. *Zuckerind.* **109**: 1014-1017.
2. Baerwald, G. and K.Y. Lee. 1989. Ueber die Zusammensetzung von Reubenmelasseschlempe hinsichtlich ihrer Verhefung. *Die Branntweinwirtschaft* **129**: 146-152.
3. Boeringer Mannheim. 1987. Methoden der enzymatischen Lebensmittelanalitik Firmenschrift, Mannheim.
4. DIN (Deutch Industrie Normen) 1980. Bestimmung des Chemischen Sauerstoffbedarfs im ueber 15 mg/l. Beuth-Verlag Berlin, Teil 41.
5. Herbert, D., P.J. Phipps, and R.E. Strange. 1971. Chemical analysis of microbial cells, p. 209-344. *In* J.R. Norris and D.W. Ribbons (eds), *Methods in Microbiology*, Vol. 5B. Academic Press, London.
6. Kreife, H. 1981. Getreide-und kartoffelbrennerei. Verlag Eugen Ulmer Stuttgart, 35-37.
7. Lee, K.Y. 1991. The growth, effect of COD-reduction, and flocculation characteristics of *Candida rugosa* in sugar beet stillages. *J. Microbiol. Biotechnol.* **1**: 207-211.
8. Lee, K.Y. and G. Baerwald. 1991. Kinetic behavior of *Candida rugosa* in the batch fermentation of sugar beet stillage; temperature dependence of growth and flocculation characteristics. *Biotechnol. Letter* **13**: 595-598.
9. Pimentel, L.S. 1980. The Brazilian ethanol programm. *Biotechnol. Bioeng.* **22**: 1889-2012.
10. Sheehan, G.J. and P.F. Greenfield. 1980. Utilization, treatment and disposal of distillery wastewater. *Water research* **14**: 257-277.
11. Weinert, I. 1978. Determination of total carbohydrate in beer. *J. Inst. Brew.* **84**: 220-223.

(Received October 20, 1994)