YEAST BIOMASS FROM SACCHARIFIED TAPIOCA PROCESSING WASTE

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Abstract

About 20 per cent of the tapioca tuber/chip processed for the manufacture of starch/sago is eliminated as fibrous residue which finds no economical use and poses disposal problems. After saccharification, it can form a source of carbon in fermentation industries. Experiments on the production of yeast biomass on saccharified waste fortified with mineral salts gave lower yields (18.20 — 44.01 per cent) when 14 different yeast strains were grown on it. Studies on optimization of media constituents for two strains increased the yield to 50.08 and 43.01 per cent respectively for **C. utilis** *3336 and* **S. cerevisiae** *RM in shake flasks. In 10 l fermentor, these strains gave specific growth rates of the order of 0.294 and 0.228 hr^{-1} respectively. The studies establish the utilization of saccharified tapioca fibrous residue as an alternate carbon source for the production of yeast biomass which will also lead to solving its disposal problems.*

Introduction

With the ever increasing population, it has become essential to look for alternate cheaper sources of protein to meet the gap between requirement and production of prote in foods. Single cell protein shows promise in this regard and therefore efforts were made throughout the world to develop technology for the production of SCP from various sources. Substrates like hydrocarbons, industrial and agricultural wastes, wood hydrolysates and grains were studied extensively. The investigations on search for newer and cheaper carbon sources are still continuing throughout the world.

Tapioca is cultivated on large scale in Southern India, especially in Kerala and Tamil Nadu. Besides using it as a food item, tapioca is extensively used for the manufacture of starch and sago by more than 200 large scale and cottage industries. Approximately 20 per cent of the tapioca processed is given out as fibrous waste residue which was formerly dried and sold as cattle feed. Due to prohibitive fuel costs, the practice of drying has been largely stopped and the industry is faced with the problem of disposal of the waste. With starch content in the range of 50-65 per cent, the waste could form an alternative cheaper substrate for producing various remunerative products such as alcohol, sugar syrup and single cell protein.

The present communication presents the data on the production of yeast protein using saccharified waste.

Materials and Methods

Saccharification. Saccharification of the waste residue by enzyme-enzyme process was carried out as per procedure reported earlier (1). The saccharified waste was found to contain about 7 per cent reducing sugars and was diluted with distilled water to desired sugar concentration.

Yeast screening. 11 strains of *Saccharomyces cerevisiae* and 3 strains of *Candida utilis* obtained from different sources were screened for the degree of biomass formation on the hydrolysate containing 10 g/l reducing sugars. The hydrolysate at pH 5.5 was suppleneted (g/l) with $(NH_4)_2SO_4$ — 2.5, $Na_2HPO_4.12H_2O$ — 3.0, $MgSO_4.7H_2O$ — 0.3, yeast extract — 0.2 and sterilized at 10 p.s.i. for 15 minutes before inoculation (1 slant/50 ml medium). The flasks were incubated on rotary shaker (230 rpm) at 28±2°C for 2 days. The biomass formed was determined and the residual sugar in the liquor was estimated by the method of Shaffer and somogyi (2).

Effect of nitrogen sources. A number of nitrogenous sources, both organic and inorganic, were investigated under similar cultural conditions. The concentration of nitrogen was kept equal (0.494 g N_2/l) in each case. After selecting the best nitrogen source, its concentration was varied to establish the optimum concentration and was used in subsequent optimization studies. Except for nitrogen source, the medium composition was same as used in screening studies.

Sugar concentration. A mineral salt Medium containing (g/l) $MgSO_4.7H_2O$—0.3, K_2HPO_4—3.0, medium concentration of the desired Nitrogen source and varying quantity of saccharified hydrolysate were used to find out optimum sugar concentration for maximum yield of the biomass under cultural conditions defined for yeast screenlng.

Effect of trace elements. The saccharified waste containing optimum sugar and nitrogen concentration as well as 10 ml/l vitamin mixture (3) was enriched with trace element mixture (4) and was studied under similar cultural conditions simultaneously with control blank and the media in which trace elements are eliminated one by one elimination technique. The other cultural conditions are described earlier.

Growth factors. The saccharified waste containing optimum concentration of sugar, nitrogen and other mineral salts as described in sacreening work were supplemented with various growth factors at 0.02% level and studied for biomass formation. The media used for *C. utilis* contained ammonium molybdate 0.000481 g/l while no trace element was used in the media used for *S. cerevisiae.* Other cultural conditions are same as reported for screening studies.

Fermentation studies. Digi-ferm laboratory fermentor designed and fabricated in the Institute was used in conducting fermentation trial at 10 level by using optimum culture parameters developed at shake-flask level. The pH of the broth was maintained at 5.5-5.8 while the temperature was maintained at 28±°2C. The optimum media contained:

	C. utilis 3336	*S. cerevisiae* RM
	Concentration in g/l	
Reducing sugars	10.0	10.0
$Na_2HPO_4.12H_2O$	3.0	3.0
$MgSO_4$	0.3	0.3
Urea	1.0	0.5
Ammonium sulphate	—	1.0
Ammonium molybdate	0.00481	—
Corn steep liquor	0.02	—
Yeast extract	—	0.02
pH	5.5-5.8	5.5-5.8

Results and Discussion

Yeast screening. A total of 11 strains of *S. cerevisiae* and 3 strains of *C. utilis* were screened and the results are given in Table 1. The dry biomass formation ranged between 1.74-4.20 g/l. *C. utilis* 3336 gave highest biomass while among the strains of *S. cerevisiae* screened, strain RM showed highest biomass formation. The per cent dry biomass yield based on sugars consumed in the range of 18.20–44.07 while the crude protein percentage in dry biomass was in the range of 41.01 — 54.01. It is interesting to note that the strains of *Candida* showed better growth on the saccharified waste medium and it may be due to the fact that the sacchairfied waste also contains pentoses which are utilised by *Candida* unlike *Saccharomyces* strains. Based on these observations, *S. cerevisiae* RM and *C. utilis* 3336 were selected for further studies on optimisation.

Effect of nitrogen sources. The saccharified waste is deficient in nitrogen and needs supplementation with nitrogen for better yields. Hence, various inorganic and organic

TABLE 1. *Comparative biomass formation by Candida and Saccha romyces strains on saccharified cassava waste.*

Strain	Dry biomass g/1	Per cent yield based on sugars consumed	Per cent crude protein in dry biomass
C. utilis NCIM 3336	4.20	44.07	49.48
C. utilis 74-63	3.82	40.34	47.13
C. utilis var. *thermopila* 74-62	3.08	32.66	44.30
S. cerevisiae alcohol strain FT-1	3.76	39.66	45.76
S. cerevisiae alcohol strain FT-18	1.74	18.20	41.14
S. cerevisiae thermophilic strain 73-84	2.62	31.33	41.01
S. cerevisiae bakery strain RM	3.76	39.70	54.01
S. cerevisiae bakery strain GR	3.42	36.08	52.72
S. cerevisiae bakery strain A	2.54	26.82	51.33
S. cercvisiae bakery strain B	2.82	29.56	47.63
S. cerevisiae bakery strain C	3.02	31.79	49.02
S. ccrevisiae bakery strain D	2.98	31.34	49.61
S. cerevisiae bokery strain E	2.70	28.01	51.30
S. cerevisiae bakery strain F	3.62	37.87	41.81

TABLE 2. *Effect of various nitrogen sources on biomass formation by yeast strains.*

Nitrogen source	Concentration of nitrogen source used g/1	*C utilis* NCIM 3336		*S. cerevisiae* RM	
		Dry biomass g/1	Per cent yield based on sugars consumed	Dry biomass g/1	Per cent yield based on sugars consumed
Sodium nitrate	3.00	0.91	23.39	0.29	2.81
Potassium nitrate	3.58	2.70	27.23	0.33	3.72
Urea	1.06	4.06	44.72	2.58	26.87
Urea+ammonium sulphate, 50 : 50 N_2	0.53 (Urea)+ 1.16 (ammonium sulphate)	3.51	35.72	4.09	40.22
Ammonium phosphate dibasic	2.32	4.74	48.02	3.70	37.17
Ammonium nitrate	1.41	3.95	40.24	3.00	29.89
Ammonium sulphate	2.33	3.48	35.36	3.08	30.73
Ammonium chloride	1.89	3.35	34.20	2.21	21.98
Ammonium citrate	3.81	5.43	54.83	3.90	37.02
Ammonium oxalate	2.51	2.44	24.60	2.14	21.16
Ammonium tartarate	3.25	3.82	38.48	3.18	31.51
Ammonium acetate	2.72	2.70	31.84	2.90	28.93
Control	0	0.80	21.39	0.69	13.17

The concentration of nitrogen sources used contained 0.494 g/1 N_2. Inoculum size was 0.33 g/1 (dry weight).

nitrogenous compounds were tested to select a suitable and economic nitrogen supplement (Table 2). Ammonium citrate, dibasic ammonium phosphate and urea were found to be the best sources of nitrogen for *C. utilis* 3336. As the first two sources are expensive, urea was selected as nitrogen sources for *C. utilis.* For *S. cerevisiae* RM, the combination of urea and ammonium sulphate (50 : 50 N_2) was the best source and such combination is also economic and hence selected.

Effect of nitrogen concentrations. The results of the studies on different concentration of nitrogen sources selected are given in Table 3. It is evident that urea at the concentration of 1 g/l is optimum for *C. utilis* 3336 while a combination of 0.5 g urea and 1 g ammonium sulphate per litre of the medium is optimum for *S. cerevisiae* RM.

Sugar concentration. Table 4 shows the effect of initial reducing sugar concentration in the medium on the degree of dry biomass formation. About 1 per cent initial reducing sugar in the medium was found to be optimum for both the strains studied. Further increase in the initial reducing sugar concentration in the medium leads to increase in degree of biomass formation but with a proportionate decrease in the percentage of sugar conversion into dry biomass. The optimum sugar concentration of 1 percent was therefore used in further work.

Effect of trace elements. The require-

TABLE 3. *Effect of various concentrations of nitrogen sources on biomass formation by yeasts.*

C. utilis NCIM 3336			*S. cerevisiae* RM			
Urea g/l	Dry biomass g/l	Per cent yield based on sugars consumed	Urea g/l	Ammonium sulphate g/l	Dry biomass g/l	Per cent yield based on sugars consumed
0.25	3.16	31.53	0.125	0.25	3.35	33.62
0.50	3.69	36.91	0.250	0.50	3.79	37.68
0.75	4.04	40.29	0.375	0.75	3.88	38.50
1.00	4.49	44.72	0.500	1.00	4.11	41.00
1.50	4.46	44.32	0.750	1.50	3.56	25.41
2.00	4.36	43.56	1.000	2.00	3.55	25.37
2.50	4.22	42.51	1.250	2.50	3.52	25.05
5.00	3.92	39.73	2.500	5.00	3.49	25.22

TABLE 4. *Effect of initial sugar concentration on biomass formation by yeast strains.*

Initial sugar concentration percentage	*C. utilis* NCIM 3336 Dry biomass g/l	*C. utilis* NCIM 3336 Per cent yield basis on sugars consumed	*S. cerevisiae* RM Dry biomass g/l	*S. cerevisiae* RM Per cent yield based on sugars consumed
0.11	0.43	45.26	3.08	40.73
0.21	0.80	44.38	0.76	40.51
0.32	1.22	44.45	1.15	40.36
0.48	2.02	44.72	1.81	39.91
0.95	4.08	44.84	3.65	40.02
1.43	4.89	34.08	3.84	28.26
1.91	5.23	28.54	3.93	21.69
2.39	6.01	26.47	4.03	17.74
2.87	6.44	23.51	4.22	15.43
3.35	7.19	22.35	4.80	14.96

TABLE 5. *Effect of trace elements on the biomass formation by yeast strains.*

Trace element	*C. utilis* NCIM 3336		*S. cerevisiae* RM	
	Dry biomass g/1	Per cent yield based on sugars consumed	Dry biomass g/1	Per cent yield based on sugars consumed
No trace element	3.97	45.75	3.91	41.68
With all trace element	3.77	43.44	3.42	36.45
Trace element omitted:				
Zinc sulphate	4.19	48.31	3.31	35.28
Copper sulphate	4.32	49.75	3.27	34.83
Ferric chloride	4.13	47.58	3.27	34.84
Ammonium molybdate	3.84	44.25	3.43	36.54
Boric acid	4.26	49.14	3.34	35.58
Manganese chloride	4.17	48.09	3.49	37.20

TABLE 6. *Effect of growth factors on the biomass formation by yeast strains.*

Growth factor	*C. utilis* NCIM 3336		*S. cerevisiae* RM	
	Dry biomass g/1	Per cent yield based on sugars consumed	Dry biomass g/1	Per cent yield based on sugars consumed
No growth factor	3.93	39.68	3.53	36.33
Yeast extract	4.92	49.37	4.19	43.01
Corn steep liquor	4.93	50.08	3.99	40.77
Whey	3.94	40.05	3.57	37.04
Beef extract	4.06	40.90	3.77	38.94
Vitamin mixture	4.89	49.11	4.10	42.12

ment of trace elements for the growth of the yeast strains was studied and the data is presented in table 5. With *S. cerevisiae* RM, the trace elements combinely or when omitted one by one showed inhibition of growth as compared to that in the medium without any trace element. In case of *C. utilis* 3336, the trace element mixture inhibited the growth and it is evident that zinc sulphate, copper sulphate, ferric chloride, boric acid and manganese chloride were inhibitory. The data indicated that the omission of ammonium molybdate from the medium resulted in lower growth.

Effect of growth factors. A significant increase in biomass yield is evident with the inclusion of vitamix mixture, yeast extract or corn steep liquor in case of both the strains (Table 6). However, the highest yield was obtained when corn steep liquor and yeast extract were added at 0.02 per cent level in case of *C. utilis* 3336 and *S. cerevisiae* RM respectively. The growth promoting abilities of whey and beef extract were poor.

Fermentation studies. Figure 1 shows the growth curves of *C. utilis* 3336 and *S. cerevisiae* RM on optimum medium in 10 1 fermentor at 30°C and at pH 5.5-5.8. The dry biomass (g/1) is plotted against fermentation time in semi-long paper. *C. utilis* 3336 and *S. cerevisiae* RM gave specific growth rates of the order of 0-294 and 0.228 hr^{-1} with generation times of 2.36 and 3.04 hr respectively. The dry biomass formed at 24 hr fermentation was 5.7 and 5.4 g/1 by *C. utilis* 3336 and *S. cerevisiae* RM respectively.

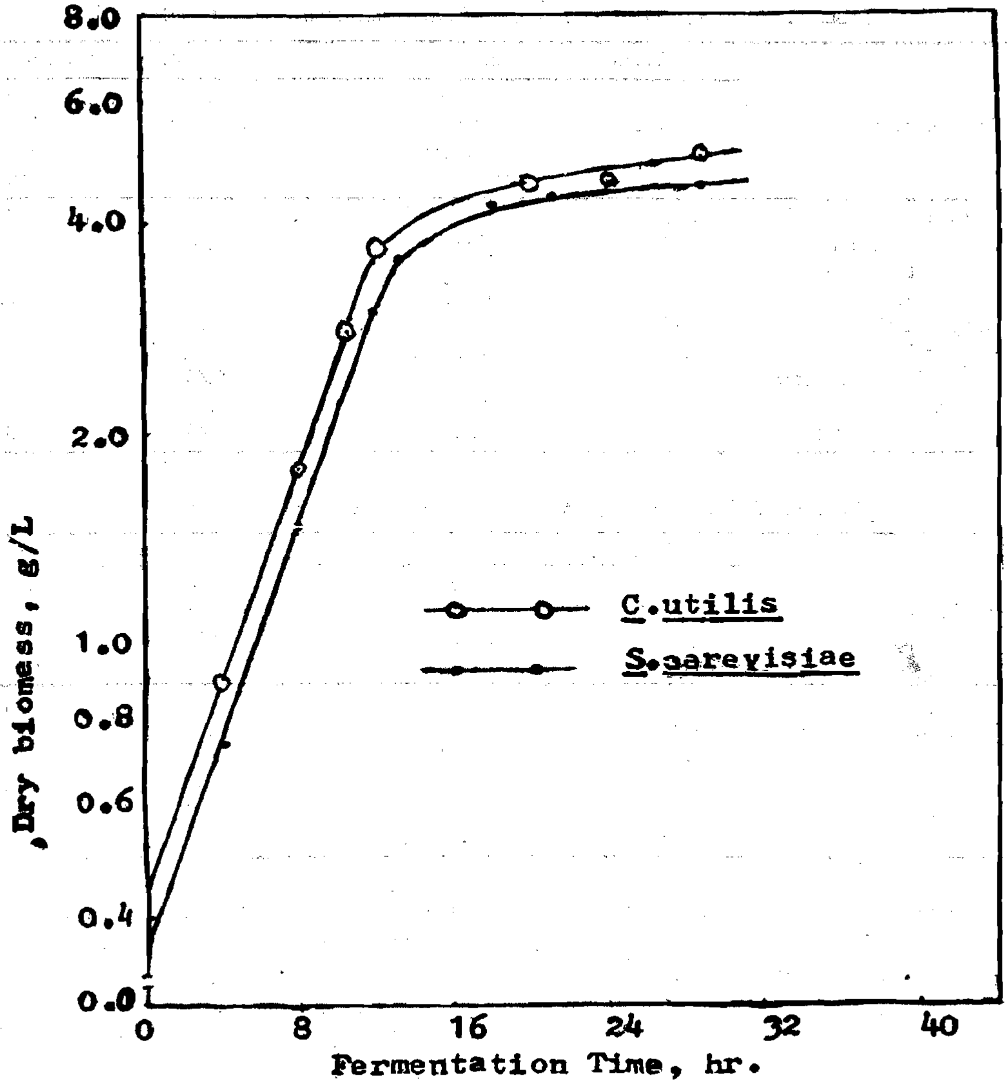

Fig. 1 Growth curve of *C. utilis* and *S. cerevisiae* on semi log paper

The demand for food yeast is on increase due to its extensive use as protein supplement and source of B group vitamins in food and pharmaceutical industries. The major assimilable carbon sources used in the production of yeast biomass are molasses (5), sulfite liquors (6), wood sugars (7), hydrolysed grains (8), fruit wastes (9), tapioca starch (10) and waste liquor from starch plant (11). The saccharified fibrous residue from tapioca processing industries is not yet used for the production of yeast biomass. The present studies indicated that it can be effectively used for the production of yeast and that the conversion of reducing sugars into yeast biomass under optimal cultural parameters is comparable to that which is normally obtained with conventional carbon sources such as molasses. Thus the fibrous waste utilization for the production of yeast biomass will not only provide an alternate cheap source of sugars but will also lead to the utilisation of waste product as well as the prevention of atmospheric polution by the waste.

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Breathes there a bard who isn't moved
when he finds hus verse is understood
And not entirely disapproved
By his country and his neighbourhood?

—Robert Frost

Ot takes all sorts of in and out-schooling
To get adapted to my kind of feeling.

—Robert Frost