CONVERSION OF CASSAVA FIBROUS WASTE RESIDUE INTO ETHANOL BY ACID—ENZYME PROCESS

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SUMMARY

The acid-enzyme hydrolysis of the starch present in cassava fibrous waste residue resulted in 96-99% conversion under optimum parameters. The hydrolysate containing about 7% reducing sugars, when fermented to obtain ethanol, gave fermentation efficiency as low as 68%. The concentration of the hydrolysate to raise reducing sugar concentration to 15% resulted in improved fermentation efficiency to the tune of 75%. The economics of the process demands the elimination of capital intensive and costly concentration step which also involves recurring expenses. These limitations could be overcome by raising the slurry concentration of the waste during hydrolysis to achieve about 15% reducing sugars in the hydrolysate.

Introduction

Cassava, one of the major crops of Southern India, is processed for the manufacture of starch and sago by more than 800 large and small scale industries[1]. Huge quantities of fibrous waste residue, popularly called as *Tippi* in Tamil Nadu, is thus generated to the tune of about 20% of the cassava roots/chips processed. For example, in and around Salem (Tamil Nadu) alone, about 600 tons of the cassava fibrous waste residue (hereafter referred to as waste) is generated per day[2]. It poses disposal problems as its drying for use in cattle feed has become uneconomical due to high fuel cost. Studies were therefore undertaken to explore the possibilities of its effective and economic utilization for the production of ethanol. The data on acid-enzyme saccharification, optimization of various parameters for fermentation to ethanol, growth characteristics of *Saccharomyces cerevisiae* on media containing saccharified waste as sole source of carbon and the economics of the process are reported in the present communication.

Materials and Methods

Saccharification: A 10% slurry of the sun-dried waste in dilute HCl solution of pH 1.4 was hydrolysed at 121°C for 1 hr[3]. After cooling to 60°C, the pH was adjusted to 4.2 with NaOH solution and crude amyloglucosidase (9000 units/ml) was added at 0.4 per cent (V/V) level. The reaction was allowed to continue at 55°C for 24 hrs with constant agitation at 200 rpm under aseptic conditions. The resulting slurry was filtered through cloth to remove cellulosic fibres and was centrifuged to obtain clear hydrolysate.

Fermentation : Studies on the production of ethanol from saccharified waste were carried out in 100 ml capacity Erlenmeyer flasks containing 50 ml medium sterilized for 20 min. at 115°C. After inoculation with *Saccharomyces cerevisiae* FT-18, the flasks were incubated at 28-30C for 5 days with intermitant manual shaking of the flask contents in the initial incubation period of 4-5 hrs. The fermented broth was distilled and the alcohol was estimated in the distillate by A.O.A.C. method[4]. Initial and residual sugars in media and the hydrolysate were estimated by Shaffer-Somogyi method[5] while dry biomass in the fermented broth was estimated as described earlier[3]. The percentages of plant and fermentation efficiencies were calculated by using standard formula[6].

Under similar cultural conditions, studies were carried out to optimize various parameters such as nitrogen and mineral salts requirement, sugar concentration, inoculum size and incubation time. The basal mineral salt medium included 0.106 g K_2HPO_4, 0.142 g Na_2SO_4 and 0.030 g $MgSO_4$ per 100 ml medium. Sugar concentration in the medium was varied from 6.92 to 30.75% while ten different organic and inorganic nitrogen sources (21.2 mg N_2/100 ml medium) were tested for selecting a suitable nitrogen source.

Results and Discussion

Saccharification : The initial trials on the saccharification of the waste residue were not satisfactory as the conversion of starch to reducing sugars was merely 35-75%. However, 98-99 per cent conversion was achieved by optimizing various parameters[3]. Table 1 shows a typical data on the saccharification of the waste by acid-enzyme hydrolysis. With about 99 per cent conversion of the starch present in the waste residue, the saccharification of the waste to glucose syrup and further fermentation for the production of ethanol will lead to the efficient utilization of the waste residue and in tackling the problems faced by the industry in its disposal. Therefore, efforts were directed on its use for the production of ethanol by yeast fermentation.

Table 1. Acid-enzyme saccharification of fosuirb waste residue : A typical data

	Parameter	Value
1.	Weight of sun-dried waste residue	1000 g
2.	Dry weight of the waste residue	870 g
3.	Hydrocyanic acid level in dry waste residue, ppm	85.7—100.6
4.	Weight of dry starch in slurry (10 per cent slurry of sun-dried waste residue)	630 g
5.	Per cent starch conversion to glucose after acid treatment at pH 1.4	
	i) based on dry waste residue	19.75
	ii) based on dry starch weight	27.28
6.	Per cent starch conversion to glucose after subsequent treatment with amyloglucosidase at pH 4.2	
	i) based on dry waste residue	71.59
	ii) based on dry starch weight	98.87
7.	Reducing sugar percentage in saccharified waste	6.92
8.	Hydrocyanic acid level in saccharified waste, ppm	0.349—0.625

Fermentation : The saccharified waste, with or without concentration, was employed as sole source of carbon for the production

Table 2. Effect of mineral salt enrichment of saccharified waste residue from cassnva processing industries on the production of alcohol

Enrichment of saccharified waste with	Sugar consumed gm/100 ml	Per cent alcohol formed (w/w)	*Fermentation efficiency	+Plant efficiency	Per cent conversion of sun dried waste to alcohol (w/w)
Nil (control)	13.073	4.925	73.727	58.643	20.976
$MgSO_4$—30 mg% + K_2HPO_4—136 mg% + Na_2SO_4—142 mg%	12.413	6.300	79.618	60.131	26.832
Urea—91 mg%	13.705	5.900	84.250	70.252	25.129
Urea—91 mg% + $MgSO_4$—130 mg% + K_2HPO_4—136 mg% + Na_2SO_4—142 mg%	14.805	6.550	86.560	77.992	27.897

$$\text{*Fermentation efficiency} = \frac{\text{g alcohol produced}}{\text{g theoretical alcohol possible from sugar consumed}} \times 100$$

$$\text{+Plant efficiency} = \frac{\text{g alcohol produced}}{\text{g theoretical alcohol possible rom sugar supplied}} \times 100$$

Table 3. Effect of various nitrogen sources on the production of alcohol from saccharified waste residue from cassava starch processing industries.

Nitrogen source	Sugar consumed gm/100 ml	Per cent alcohol formed (w/w)	Fermentation efficiency	Plant efficiency	Per cent conversion of sun dried waste to alcohol (w/w)
NH_4NO_3	18.375	6.075	64.699	59.444	21.264
NH_4Cl	18.425	6.105	64.842	59.737	21.369
$(NH_4)_2C_2O_4.H_2O$	18.275	6.105	65.374	59.737	21.369
$NH_2.CO.NH_2$	18.250	6.200	66.483	60.667	21.701
$(NH_4)_2HPO_4$	17.875	6.125	67.056	59.933	21.439
$CH_3.COO.NH_4$	18.300	6.105	65.285	59.737	21.369
$(NH_4)_2HC_6H_5O_7$	17.988	6.105	66.417	59.737	21.369
$(NH_4)_2SO_4$	17.900	6.050	66.143	59.199	21.176
$NaNO_3$	16.875	5.775	66.971	56.508	20.213
$(NH_4)_2HPO_4$ + $(NH_4)_2SO_4$	18.200	6.025	64.784	58.955	21.088

Table 4. Effect of sugar concentration in saccharified waste on degree of alcohol formation.

Initial sugar g/100 ml	Sugar consumed g/100 ml	Per cent alcohol formed (w/w)	Fermentation efficiency	Plant efficiency	Per cent conversion of sun dried waste of alcohol (w/w)
6.920	6.635	2.31	68.060	65.326	23.366
11.000	10.525	3.84	71.360	68.315	24.437
14.770	14.090	5.44	75.528	72.077	25.782
21.750	18.925	6.86	70.898	61.722	22.078
24.675	18.645	5.90	61.873	46.792	16.738
30.750	15.980	4.92	60.251	31.311	11.200

Table 5. Effect of inoculum size on the production of alcohol

Inoculum size cells/flask	Sugar consumed* g/100 ml	Per cent alcohol formed (w/w)	Fermentation efficiency	Plan efficiency	Per cent conversion of sun dried waste to alcohol (w/w)
4.84×10^6	13.963	5.766	80.806	75.225	25.781
9.68×10^6	14.191	6.259	86.318	81.657	27.986
24.2×10^6	14.595	6.057	81.209	79.022	27.082
96.8×10^6	14.726	5.517	73.313	71.977	24.668

*Initial sugar was 15 g/100 ml medium

of ethanol by *Saccharomyces cerevisiae* TF-18 and various cultural parameters were standardized to obtain higher yields of ethanol.

Fortification with mineral salts and nitrogen: The studies indicated improvements in fermentation efficiences when the saccharified waste was fortified with mineral salts and nitrogen (Table 2). Different nitrogen sources, when tested, resulted in more or less similar fermentation efficiencies (Table 3). Among them, urea is the preferred source of nitrogen as it is cheapest and contains more nitrogen per g of the compound. It's use is also characterized by elimination of the need for the large quantities of alkali for maintenance of the pH of the fermentation broth during the course of the fermentation. Similar results regarding supplementation of cassava root pulp with mineral salts and nitrogen are known[7,9].

Effect of sugar concentration : It was observed that the fermentation efficiencies increased with the increase in initial sugar concentration from about 7 to 15%. However this was followed by a decrease when sugar concentration is beyond 15% (Table 4). Though higher fermentation efficiency was observed with initial sugar concentration at about 15%, the use of initial sugar at about

7% is preferred from economic point of view even when it leads to slightly lower fermentation efficiency. The saccharified waste contains about 7% sugars and its concentration to raise sugar level to about 15% will involve the expenditure of Rs. 4.89 per litre of additional ethanol produced on steam cost alone apart from the extra capital investment estimated at Rs. 6.0 lakhs on triple effect evaporator and additional steam generation capacity[10]. Other justifications do not permit the use of mollases to raise the sugar contents of the saccharified waste[10].

Effect of inoculum size : The results of the different inoculum sizes on ethanol production are presented in table 5. It is interesting to note that the fermentation efficiencies increased with the increase in inoculum size from 4.84×10^6 to 9.68×10^6 but decreased with further increase in inoculum size. The inoculum size of 9.68×10^6 cells per flask containing 50 ml fermentation medium is optimum for the production of ethanol from saccharified waste.

Relation between sugar consumption and alcohol fermentation : Fig. 1 shows the time course of biomass formation, ethanol production and sugar consumption in flask containing 50 ml medium. After a lag phase of about 6 hrs. the yeast grew exponentially upto 72 hrs. followed by a phase of declined growth. The rate of alcohol production was directly proportional to the rate of sugar consumption. The degree of alcohol production and biomass formation started declining after 120 hrs. The initial pH of 4.5 gradually decreased to 4.0 in the first 24 hrs of fermentation and remained constant at 4.0 throughout the rest of the fermentation period. The trend of ethanol production, biomass formation and sugar consumption is similar to that on other conventional carbohydrate substrates.

Feasibility of the process : The fibrous waste residue generated in the manufacture of starch and sago from tapioca tubers/chips demands adequate treatment due to its high BOD and COD levels before it could be discharged in the streams and rivers. To offset the costs involved in waste treatment, attempts were made by workers to utilize it for various purposes or to recover byproducts[11-14] but proved to be uneconomical due to the high transportation costs or low value products. The intensive efforts for effective economical utilization of the waste in CFTRI have established that the saccharification of the waste residue to glucose syrup[3] or the conversion of enzyme-enzyme hydrolyzed waste into alcohol[10] offers definite economic advantages to the starch industry. The present studies revealed the possibilities of production of ethanol from the waste saccharified by acid-enzyme hydrolysis which works out to be more economical as compared to enzyme-enzyme hydrolysis process. The starch granules in the waste are located in the intact root cells that were not ruptured during the rasping process. Consequently, the use of enzyme-enzyme process for aasccharification of the starch present in the waste demands drastic conditions for releasing the starch granules from the root cells. This invariably involves pressure cooking of the slurry at 121°C for as long as 1 h and thus increases the expenditure on the saccharification. The liquifaction and limited saccharfaction of the waste with α-amylase involve about 2-3 h against 15 min required for the same with the use of acid. Moreover, the over-all conversion of starch into reducing sugars is more or less equal in both the cases.

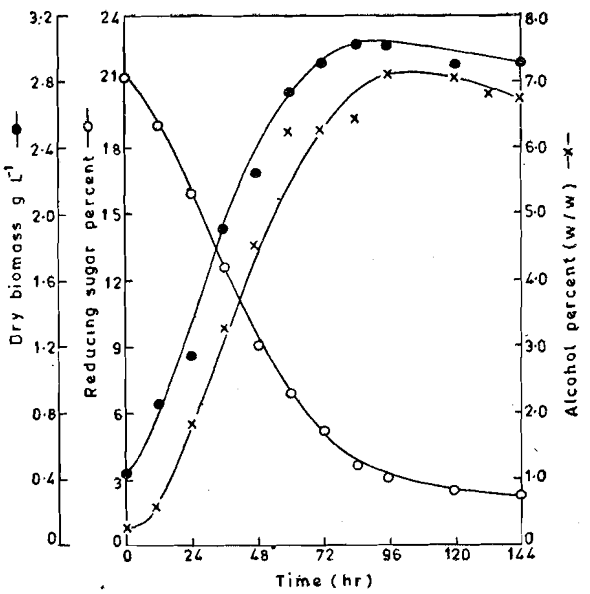

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Fig. 1. Relationship between ethanol production, biomass formation and sugar consumption.

Recently, the know-how for the manufacture of potable alcohol from tapioca flour, developed by CFTRI, is released to six industries One of them have produced lakhs of litres of alcohol and other entrepreneurs are under various stages in the implementation of the process. These developments took place due to current crisis in the availability of molasses. Incidently, unusually high increase in the cost of tapioca chips/flour and scarcity of tapioca was observed recently probably due to the off-shoot of its utilization in potable alcohol production. In this connection, the use of tapioca fibrous waste for alcohol production will relieve the demand of tapioca tubers for more appropriate utilization in manufacture of starch, sago and as the staple food.

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