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Ethanol Production from Sago Waste Using *Saccharomyces cerevisiae* Vits-M1

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Abstract: The present study deals with the biotechnological production of ethanol from sago waste materials. As petroleum has become depleted, renewable energy production has started to gain attention all over the world, including the production of ethanol from sago wastes. In our research we have standardized the production of ethanol from sago wastes using *Saccharomyces cerevisiae* strain isolated from molasses. The production of ethanol was carried out by means of simultaneous saccharification with acids, followed by fermentation. The yeast strains were isolated from either batter or molasses and the taxonomy was studied by phenotypic characters in comparison with the standard strain *Saccharomyces cerevisiae* MTCC 173. Among the two isolated strains, *S. cerevisiae* VITS-M1 isolated from molasses showed better survival rate in different sugars such as glucose, sucrose, maltose and galactose except lactose; it also showed better survival rate at high ethanol concentration and at acidic pH. The saccharification process of sago liquid waste and solid waste was standardized using hydrochloric acid and sulphuric acid under different treatments. The fermented product, ethanol was distilled using laboratory model distillation unit and measured qualitatively using gas chromatography in comparison with the standard analytical grade ethanol. The overall experimental data indicates that the sago liquid waste yielded more ethanol by simultaneous saccharification with 0.3N HCl and 0.3N H_2SO_4 and fermentation with the *S. cerevisiae* VITS-M1 isolated from molasses.

Key words: Ethanol, fermentation, saccharification, *Saccharomyces cerevisiae* VITS-M1, sago wastes

INTRODUCTION

Ethanol, also known as grain alcohol, can be made from any sugary (or) starchy agricultural crop such as corn, barley, wheat or from cellulosic biomass such as wood, paper pulp or agricultural waste (Thomas and Kwong, 2001). Large quantities of ethanol are used as solvent and chemical feed stock in various industries. Although most of the industrial ethanol is currently produced from the catalytic conversion of ethylene, considerable research has been focused on fermentation based ethanol production from various renewable biomass sources (Ladisch and Svarczkopf, 1991; Worley *et al*., 1992; Sosulski and Sosulski, 1994; Ingledew *et al*., 1995; Wang *et al*., 1997). During energy crisis in 1970s, there were numerous research efforts directed towards the development of alternative energy sources, and ethanol production from agricultural products (Nagashima *et al*., 1984). Consequently, this technology has been successfully used in Brazil where a large number of cars are run on either Gasohol (76% gasoline and 24% ethanol) or pure ethanol. Furthermore, the use of lead in gasoline has been prohibited in many countries and ethanol is now used in place of lead as an octane enhancer. The world ethyl alcohol production has reached about 51,000 mL (Renewable Fuels Association, 2007), being the USA and Brazil the first producers. In average, 73% of produced ethanol worldwide corresponds to fuel ethanol, 17% to beverage ethanol and 10% to industrial ethanol.

Tapioca cassava (*Manihot esculenta crantz*) was introduced in India during the later part of the 17thcentury by the Portuguese living in the state of Kerala. Cassava, a tuber crop which is also known as manioc, sagu, yucca and tapioca is one of the most potential root crops with great potentials for bioethanol production. It is one of the most important subsistence food and industrial crop for the developing countries (Balagopalan *et al*., 1988). Cassava starch costs 15-30% less to produce per acre than corn starch making cassava an alternate and strategic source of renewable energy, raising the possibility that it could be used globally to alleviate dependence on fossil fuels (Hankoua and Besong, 2009). Tapioca is valued for its starch content and mainly used by sago industries. Recently cassava has emerged as the primary starch based feed stock for future fuel ethanol production in Africa and Asia (Drapoch, 2008). It has been vital for many industries with the application of biotechnology, especially in the fermentation industries. There are about 800 sago and starch units situated throughout the state of Tamilnadu, India. The sago industry is spread over Salem, Namakkal, Dharmapuri, Erode, Trichy and Perambalur districts of Tamilnadu, India. Cassava is one of the main sources for ethanol production (Lindeman and

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Rocchiccioli, 1979). The yield of ethanol per ton is higher from cassava (150 liters per ton of fresh root) than from sugarcane 48 liters per ton (Balagopalan *et al*., 1988). The yeast *S. cerevisiae* is an environmentally safe microorganism commonly used in industries to ferment glucose into ethanol. It has been used since ancient times to make beer and wine. *S. cerevisiae* IFO 2363 is one of the most suitable strains of yeast for ethanol production (Wada *et al*., 1981).

The aim of the present investigation was to isolate *S. cerevisiae* from molasses and batter, identification of elite strain for fermentation, selection of best saccharification method and standardization of the saccharification method for recovery of ethanol from the sago wastes.

METHODOLOGY

Sample collection and isolation of fermentative yeast: Sago waste samples were collected in sterile containers during August to December 2007 from Salem Sago Industry, Salem, Tamilnadu, India and stored at 4ºC. *S. cerevisiae* was isolated from two different sources such as molasses and batter.

One gram of the sample from each source was inoculated into 5 ml yeast extract malt extract (YM) broth in test tubes. The tubes were incubated at 28±1ºC for 48 h. (Gupta *et al*., 1994). After incubation, yeast cultures were isolated from YM broth by dilution plate technique. One ml of incubated YM broth was taken and serially diluted upto 10^6 dilutions in sterile water and 1 ml of 10^6 diluted suspensions was spread over yeast extract peptone dextrose (YEPD, pH 3.5) agar plates and incubated at 28±1ºC for 48 h. After incubation, observed yeast colonies were subcultured on YEPD agar plates for purification. Purification was repeated twice and a single colony was selected. The strain *Saccharomyces cerevisiae* strain MTCC 173 obtained from Microbial Type Culture Collection, IMTECH, Chandigarh was used for comparison.

Maintenance of strain: The culture was grown at 28±1ºC for 48 h in a medium containing 1% yeast extract, 2% peptone, 2% glucose and 2% agar. The culture was stored at 4ºC and sub cultured at regular intervals in order to maintain viability. For a long-term storage, stock cultures were maintained in 20% glycerol at -80ºC.

Characterization of the selected yeast isolates: Yeast isolates were identified based on the morphological characters (Kreger-Van Rij, 1984; Mesa *et al*., 1999) and physiological characters.

Morphological characterization: The isolated strains were inoculated at 1% level in 100 mL of sterile glucose yeast extract peptone broth in 250 mL conical flask, incubated at 28±1ºC for 48 h and examined for vegetative cells shape and budding pattern under phase contrast microscope (Hund, Germany).

Physiological characterization: The isolated strains along with standard *S. cerevisiae* strain (MTCC 173) were screened for their growth in different carbon sources as described by Wickerham (1948). Tolerance to sugar concentration, ethanol, temperature and pH was performed by using the standard protocols (Bowman and Ahearan, 1975).

Carbohydrate fermentation test: Yeast fermentation broth medium was used for identification of yeast based on the fermentation of various carbohydrates (Glucose, Maltose, Sucrose and Lactose). A Durham tube was used to detect gas produced during fermentation. In this test, tubes of media, each containing a specific carbohydrate was inoculated with a yeast isolate. The color change in the medium from purple to yellow indicates the acid production.

Tolerance to glucose concentration: Tolerance of the strains to different levels of glucose concentration was accessed. Different concentration of glucose (10, 15, 20, 25 and 30%) was added in YM broth and sterilized. The flasks were then cooled to room temperature and 1 mL of 24 h old yeast culture (approximately 10^5 cells/mL) was inoculated aseptically. The flasks were then incubated at 28±1ºC for 7 days. After incubation, the population was estimated by serial dilution and plating.

Tolerance to ethanol: Tolerance of yeast cultures to ethanol was tested in comparison with the standard strain *S. cerevisiae* MTCC 173. One ml of 24 h old culture (approximately 10^5 cells/mL) was inoculated in 100 ml YM broth adjusted to 20º Brix with glucose and subjected to different concentrations of ethanol (0, 8, 10, 12, 14, 16 and 18% v/v) to the YM broth and incubated at 28±1ºC for 7 days. After incubation, the population was estimated by serial dilution and plating.

Tolerance to temperature: Tolerance of yeast isolates to different temperature level was tested in comparison with the standard *S. cerevisise* MTCC 173. One mL of 24 h old yeast culture was inoculated aseptically into 100 mL YM broth with 20˚ Brix and incubated at 10, 20, 30 and 40ºC for 7 days. After incubation the population was estimated by serial dilution and plating as described earlier.

Tolerance to pH: Tolerance of yeast isolates to different pH levels was tested in comparison with the standard *S.cerevisiae* MTCC 173. In 100 mL YM broth containing 20º Brix sugar the pH was adjusted to 2.5, 3.5, 4.5 and 5.5

using 5% citric acid and 0.1Nsodium hydroxide. One mL of 24 h old yeast culture (10^5) was inoculated in the pH adjusted sterilized YM medium and incubated for 7 days. After incubation the population was estimated by serial dilution and plating.

Substrate for fermentation:

Sago wastes: Sago waste samples were taken in an Erlenmeyer flask with different concentrations of acids (0.1 to 0.5N) and maintained at 120ºC at different time intervals (60, 120 min) for studying the release of sugar (saccharification).

Treatment details:

Substrates: The sago wastes sample was saccharified with HCl and H_2SO_4 at different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5N) and allowed for fermentation with yeast *Saccharomyces cerevisiae.*

Estimation of reducing sugar: The reducing sugar concentration in the samples during saccharification of the substrates was estimated as per the method specified by Nelson and Somogyi (1952).

Twenty five ml of sample from the various treatments was withdrawn at regular intervals of 60,120 min from saccharified materials (using acids) cooled to room temperature and then filtered through muslin cloth. The filtrate was centrifuged at 3000 rpm for 15 min. The clear supernatant of the hydrolysate was taken for estimation of reducing sugar. Sugar was extracted with 80% ethanol twice (50 mL each time). The supernatant was evaporated by keeping in water bath at 80ºC and the residue was dissolved by adding 10 ml water. Aliquots of (0.1 or 0.2 mL) each sample was pipetted out in the test tubes and simultaneously 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard were pipetted out into test tubes. Volumes of samples and standards were made up to 2 mL with distilled water. To 1 mL of distilled water blank, 1ml of alkaline copper tartarate reagent was added to each tube. The tubes were placed in boiling water for 10 min and after cooling 1 mL of arsenomolybdate reagent was added. Finally, the volume was made up with distilled water to 10 mL and left for 10 min. Absorbance of blue color was read at 620 nm using Spectrophotometer and the values were plotted in a graph against concentration. Reducing sugars present in the sample were calculated and expressed in terms of percentage.

Saccharification: Saccharification of the waste materials was done as per the procedure described by Balagopalan *et al.* (1979).

Hydrochloric acid saccharification: Hydrochloric acid was prepared at different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5N), stored in an amber colored bottle and used for saccharification of the sample. The sample of about 100 mL sample was treated with hydrochloric acid at various concentrations (0.1, 0.2, 0.3, 0.4 and 0.5N). The acid sample mixture was autoclaved at 120ºC for 60 and 120 min for saccharification. The samples under different treatments were withdrawn simultaneously at different time intervals for the estimation of reducing sugar.

Sulphuric acid saccharification: Sulphuric acid was prepared at different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 N) and used for saccharification of substrates as described in 2.7.1. Samples were withdrawn from the treatments at different intervals for the estimation of reducing sugars. After saccharification, the samples are cooled to inoculate yeasts to carry out fermentation.

Screening yeast isolates for ethanol production: The yeast isolates were screened for its efficiency in comparison with the standard *Saccharomyces cerevisiae* MTCC173 based on its tolerance characters such as, pH, temperature, ethanol and glucose in YM medium. The best performed strain from molasses was selected and used for the fermentation process.

Fermentation conditions: Fermentation conditions were performed as described by Ciani and Maccarelli (1998). After saccharification, the substrates were allowed to ferment with yeast strain *S. cerevisiae.* The set up was left under aerobic condition for 2 days for multiplication of cells and then anaerobic condition for 16-18 days at 28±1ºC. During fermentation, the samples were withdrawn at a regular interval of 0, 5, 10, 15 and 20^{th} day and the yeast population was estimated by serial dilution and plating method. The substrates which were saccharified with different time periods and kept for different period of fermentation (5, 10, 15 and 20 days) in replications were taken and filtered through muslin cloth. The extracts recovered were distilled at 79ºC for the collection of ethanol.

Fermentation parameters: The saccharified substrates were allowed for fermentation for 15 days as described previously. During that period, the initial and final pH, reducing sugar, titrable acidity, and volatile acidity were recorded. Titrable acidity was estimated as described by Association of Official Analytical Chemists (AOAC, 1991). Volatile acidity was estimated using the method of Rodriguez *et al.* (2001).

Titrable acidity: Titrable acidity was estimated for the sample during the initial and final stages of fermentation as described by Association of Official Analytical Chemists (AOAC, 1991). To 1 mL of phenolphthalein indicator 200 mL of distilled water was added. Five mL of fermented sample was transferred volumetrically and titrated against 0.05 N standard NaOH. The end point was

the appearance of pink color which coincided with pH 8.2. The titrable acidity was calculated and expressed in g/L of tartaric acid.

$$\text{Titrable acidity (g/L of tartaric acid)} = \frac{\text{(Volume of base) (N base) (0.075) (1000)}}{\text{Sample volume (mL)}}$$

Volume of base : Volume of NaOH used in mL
N base : Normality of NaOH

Volatile acidity: Volatile acidity was estimated for the sample during the initial and final periods of fermentation as described by Rodriguez *et al*. (2001). To 10 mL of 72 h fermented material 200 mL of deionized water was added in the boiling chamber of the distillation apparatus. One hundred distillate was collected and 2-3 drops of phenolphthalein indicator was added to the distillate and titrated using standard sodium hydroxide (0.1N NaOH) upto appearance of pink color as end point. The volume of NaOH used for titration was recorded and the volatile acidity was calculated and expressed in g/L acetic acid.

$$\text{Volatile acidity (g/L acetic acid)} = \frac{\text{(Volume of base) (N base) (0.060) (1000)}}{\text{Sample volume (mL)}}$$

Volume of base : Volume of NaOH used in ml
N base : Normality of NaOH

Ethanol fermentation: The pretreated sample obtained after the saccharification using acid, was taken, adjusted to PH 5.0 using 0.1 N NaOH and the fermentation was carried out by *Saccharomyces cerevisiae*. Fermentation was carried out in samples for 5-20 days and the yeast populations at different time periods were estimated.

Recovery of ethanol: The fermented sago waste was distilled to recover the total ethanol yield by using laboratory model distillation unit after filtering through muslin cloth. The ethanol was distilled and estimated qualitatively using gas chromatography and expressed in % (v/v) of the substrate.

RESULTS AND DISCUSSION

Isolation and selection of yeast isolates: Ethanol fermentation and recovery were not only depending on the substrate used, but also depends mainly on the efficiency of yeast strain to convert the reducing sugar to ethanol. In the present study; an attempt was made to evaluate sago wastes for ethanol production by isolating elite yeast strains from two sources and optimizing conditions for simultaneous saccharification and fermentation. The yeast strain isolated from molasses was more prominent than

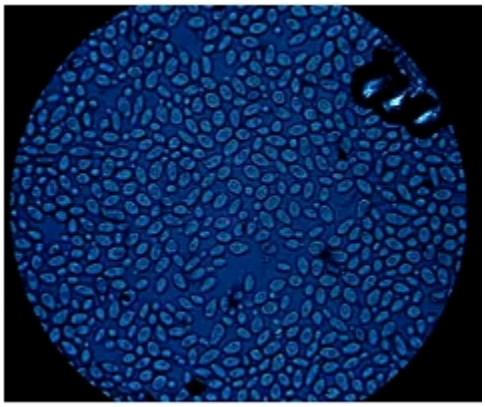

Fig. 1: Light microscopic view of *Saccharomyces cerevisiae* VITS-M1 grown in YM at 28ºC for 5 days

Table 1: Carbohydrate fermentation test

Carbohydrate	Molasses	Batter	*Saccharomyces cerevisiae* MTCC 173
Glucose	+++	+++	+++
Maltose	+	+	+
Sucrose	++	++	++
Lactose	-	-	-
Galactose	+	+	+

+++: Good growth; ++: Medium growth; +: Poor growth; -: No growth

that of the strain isolated from batter. Hence the molasses-isolated strain was selected for further studies and the strain was designated as *S. cerevisiae* VITS-M1. Monte *et al*. (2003) observed earlier that *S. cerevisiae* isolated from molasses showed higher ethanol production rate at different dilutions of sugar-cane molasses than the other strains of the yeast. Similarly, Bowman and Ahearan (1975) have characterized an *S. cerevisiae* strain with fast residual sugar fermenting ability. Recent studies have clearly suggests the impact of *S. cerevisiae* isolated from molasses in the production of ethanol from the sago wastes. The strain *S. cerevisiae* VITS-M1 was deposited in the Microbial Culture Collection Centre, SBST, VIT University.

Morphological characterization of the yeast isolates: The yeast isolated from different sources such as molasses and batter were identified based on their colony morphology, vegetative cell structure, and spore structure. Most of the isolated yeast colonies were raised, smooth and glossy. After staining with lacto phenol cotton blue, vegetative cells, shape, budding pattern were observed under the microscope and was compared to standard *S. cerevisiae* MTCC 173 (Fig. 1).

Physiological characterization of yeast isolates: Yeast isolated from molasses and batter was able to utilize various sugars such as glucose, maltose, sucrose, galactose but not lactose, and was compared to standard *S. cerevisiae* MTCC 173 (Table 1). Growth is indicated

Table 2: Growth of yeast isolates at different Glucose concentration, Ethanol concentration, Temperature and pH in YM medium

	Population (CFUx10^5/mL) yeast isolates		
	Molasses	Batter	*Saccharomyces cerevisiae* MTCC 173
Glucose (%)			
10	66	47	78
15	81	58	92
20	123	111	138
25	99	72	127
30	48	35	109
Ethanol (%)			
8	79	64	85
10	76	58	81
12	74	55	82
14	71	55	80
16	72	54	78
18	24	19	30
Temperature (ºC)			
10	6	5	7
20	27	18	29
30	94	77	99
40	73	59	81
pH			
2.5	7	5	6
3.0	9	6	10
3.5	18	11	19
4.0	28	19	25
4.5	59	46	63
5.0	91	83	97
5.5	88	81	94

by turbidity in the broth medium. Fermentation of carbohydrate is indicated by accumulation of gas in the Durham tube and change of the indicator from purple to brick red.

Growth of yeast isolates at various concentrations of glucose in YM medium: Growth of yeast isolates in different glucose concentrations in YM medium is given in Table 2. The results indicated that the two isolates have maximum population at 20% glucose concentration. The yeast isolated from molasses and batter recorded maximum population at 20% glucose concentration with the maximum population of 123, 111x10^5 CFU/mL. The reference culture showed significantly higher growth at 20% glucose concentration (138x10^5 CFU/mL) as compared to other two isolates.

The isolates were tested for sugar (glucose) tolerance at different concentration and the results showed pronounced growth at 20 BRIX. Tilak *et al.* (1974) showed that at the concentration of 25 BRIX, yeast cells showed 71% viability with the ethanol content of 9.5%. Further addition of sugar accounted for a lower rate of metabolic activity and also at higher concentrations of sugars, substrate inhibition may affect the rate of glycolysis restricting the energy supply for the survival if yeast cells. This deficiency with respect to energy may ultimately cause death to the cell.

Growth of yeast isolates at different ethanol concentrations in YM medium: The growth of yeast isolates in different ethanol concentrations is given in Table 2. Yeast isolated from molasses showed maximum population (79x10^5 CFU/mL) at 8% concentration and gradually decreased at higher concentrations. The isolate from molasses recorded maximum tolerance upto 16% ethanol with a maximum population of 72x10^5 CFU/mL similar to that of standard strain *S. cerevisiae* MTCC 173 (78x10^5 CFU/mL) whereas the other strain showed poor tolerance. Gray (1941) observed that the yeast *S. cerevisiae* tolerated ethanol concentration of 15% and effectively utilized 97.5% available glucose in the medium with a population of 62x10^5 CFU/mL. Above these concentrations the effective utilization of sugar reduces gradually with decline in population. The presence of phospholipids and ergosterol in the yeast which is accumulated during its growth is the reason for its ethanol tolerance.

Growth of yeast isolates in YM medium at various temperatures: The temperature tolerance of the yeast isolates are given in Table 2. Yeast isolated from molasses showed higher population (94x10^5CFU/mL) at 30ºC, followed by the yeast isolate from batter (77x10^5 CFU/mL). However the standard culture *S. cerevisiae* showed the maximum population of 99x10^5 CFU/mL. Beyond 30ºC the growth of isolated population declined.

Growth of yeast isolates in YM medium with different pH: The growth of yeast isolates at different pH is given in Table 2. The isolates from molasses and batter recorded

Table 3: Effect of different concentrations of HCl and H_2SO_4 on saccharification of sago waste at different time intervals

	Reducing sugar (%)									
	HCl and H_2SO_4 concentrations (N) at different time intervals									
	60 min					120 min				
Sago waste	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5
Liquid waste	54.4	45.3	56.6	49.7	60.2	56.2	57.4	51.1	53.1	49.9
	58.4	50.3	63.2	54.9	69.2	62.0	63.0	56.0	56.0	52.4
Solid waste	14.2	10.6	14.5	11.4	14.8	13.2	14.2	12.7	12.4	10.9
	13.0	14.3	15.0	15.0	20.0	16.4	14.0	13.6	12.0	12.4

Upper values - HCl; Lower values - H_2SO_4

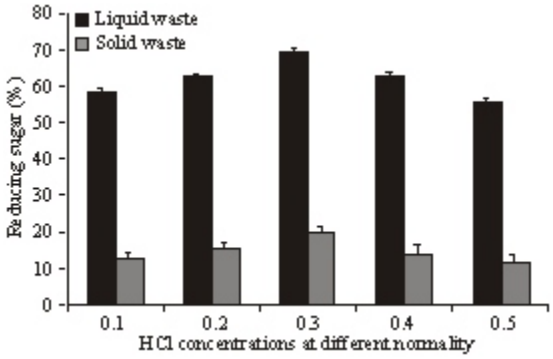

Fig. 2: Effect of different concentrations of HCI on saccharification of sago wastes at 120 min

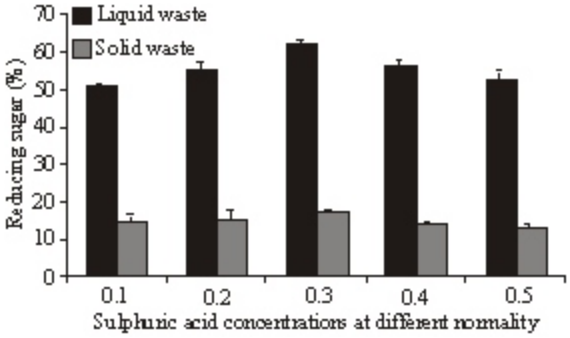

Fig. 3: Effect of different concentrations of H_2SO_4 acid on saccharification of sago wastes at 120 min

maximum population ($91x10^5$ CFU/mL) and ($83x10^5$ CFU/mL) next to the standard strain ($97x10^5$ CFU/mL). Above pH 5.0 yeast populations declined. Linden *et al*. (1992) carried out fermentations, with *S. cerevisiae* at pH 4.5, 5.0, 5.5, 6, 7 and 8 and found that the optimal pH for ethanol production was around 5.5.

Effect of different concentrations of HCl on saccharification of sago waste at different time intervals: Effect of different concentrations of HCl on saccharification of sago waste was tested with different time intervals of 60 and 120 min. At 60 min of incubation the maximum amount of reducing sugar was observed at 0.3N HCl concentration (Table 3). Sago liquid waste recorded highest reducing sugar percentage (60.2%) followed by solid waste (14.8%) in 0.3N HCl but at 0.5N HCl their reducing sugar content was 53.1 and 12.4%, respectively (Fig. 2). At 120 min after incubation, similar trend was observed at 0.3 N HCl followed by 0.2 and 0.4 N HCl. On comparison the different incubation periods of saccharification with different concentrations of HCl, 120 min incubation period with 0.3 N HCl recorded maximum reducing sugar content (69.2%) for the sago liquid waste followed by solid waste (20%), respectively.

Fermentation characters of the yeast isolated from molasses on sago waste after HCl saccharification at 120 min: During fermentation process various parameters such as pH, reducing sugar percentage, titrable acidity, and volatile acidity were assayed (Table 4). The final pH of the sago waste was about 5.0, and reduction in the reducing sugar content was recorded. The sago liquid waste had maximum amount of reducing sugar at the beginning (69.2%) followed by solid waste (20%) which decreased gradually to 7.1 and 6.5% due to fermentation. The titrable acidity and the volatile acidity increased with the fermentation of the sago waste.

Effect of different concentrations of H_2SO_4 on saccharification of sago waste at different time intervals: Effect of different concentrations of H_2SO_4 on saccharification of sago waste was tested at different time intervals of 60 and 120 min by estimating the reducing sugar percentage (Table 3). At 60 min of incubation, maximum reducing sugar was observed in 0.3 N H_2SO_4 for liquid waste (56.2%) followed by solid waste (13.2%), respectively.

At 120 min incubation, sago liquid waste recorded maximum saccharification of about 62.0% reducing sugar followed by solid waste 16.4%, respectively (Fig. 3). The results indicated that saccharification with 0.3 N H_2SO_4 for 120 min was maximum for the sago waste.

Fermentation characters of the yeast isolated from molasses on sago waste after H_2SO_4 saccharification at 120 min: Various parameters such as pH, reducing sugar content, titrable acidity and volatile acidity were recorded during fermentation (Table 4). The final pH of the liquid waste and solid waste was about 5.1. The sago liquid waste had maximum amount of reducing sugar (62.0%) at the beginning and decreased to 6.8% followed by solid waste from 16.4 to 5.5%. Titrable acidity and volatile acidity increased with the fermentation of sago waste.

Effect of different saccharification periods of 0.3N HCl on the ethanol yield of sago waste, inoculated with yeast isolated from molasses: Saccharification period at 120 min recorded maximum ethanol yield for sago waste treated with 0.3 N HCl (Table 5). At 60 min, liquid waste yielded ethanol yield of about 13.7% followed by solid waste of about 7.9%. The maximum yield of ethanol for liquid waste at 120 min is about 15.6% followed by solid waste 8.8%. Moreover, at all saccharification periods viz., 60 and 120 min, the ethanol was found to increase upto 15 days and after that gradual reductions in ethanol yield was observed. Among the varying saccharification periods with 0.3 N HCl, the ethanol yield increased linearly with the time of incubation upto 15 days and reduced afterwards.

Table 4: Fermentation characters of the yeast isolate (Molasses) on sago waste with 0.3 N HCl and 0.3 N H_2SO_4 saccharification at 120 min

		Reducing sugar (%)		Titrable acidity (g/L of tartaric acid)		Volatile acidity (g/L of acetic acid)	
Sago waste	Final pH	Initial	Final	Initial	Final	Initial	Final
Liquid Waste	5.0	69.2	7.1	4.98	5.72	0.54	0.65
	5.1	62.0	6.8	5.29	5.91	0.52	0.61
Solid Waste	5.0	20.0	6.5	3.90	4.10	0.31	0.42
	5.1	16.4	5.5	4.31	4.60	0.34	0.38

Upper values - 0.3N HCl; Lower values - 0.3N H_2SO_4

Table 5: Effect of different saccharification periods of 0.3N HCl and 0.3N H_2SO_4 on the ethanol yield of sago waste, inoculated with yeast isolated from molasses

	Ethanol yield in percentage (v/v)							
	Saccharification period (60 min)				Saccharification period (120 min)			
Sago waste	5th day	10th day	15th day	20th day	5th day	10th day	15th day	20th day
Liquid waste	10.8	11.4	13.7	13.5	11.8	13.8	15.6	14.7
	10.4	11.2	13.1	13.0	11.5	13.0	15.0	14.2
Solid waste	6.2	7.1	7.9	7.6	6.4	7.6	8.8	8.1
	5.8	6.4	7.3	7.0	6.0	6.6	7.5	6.9

Upper values - 0.3 N HCl; Lower values - 0.3 N H_2SO_4

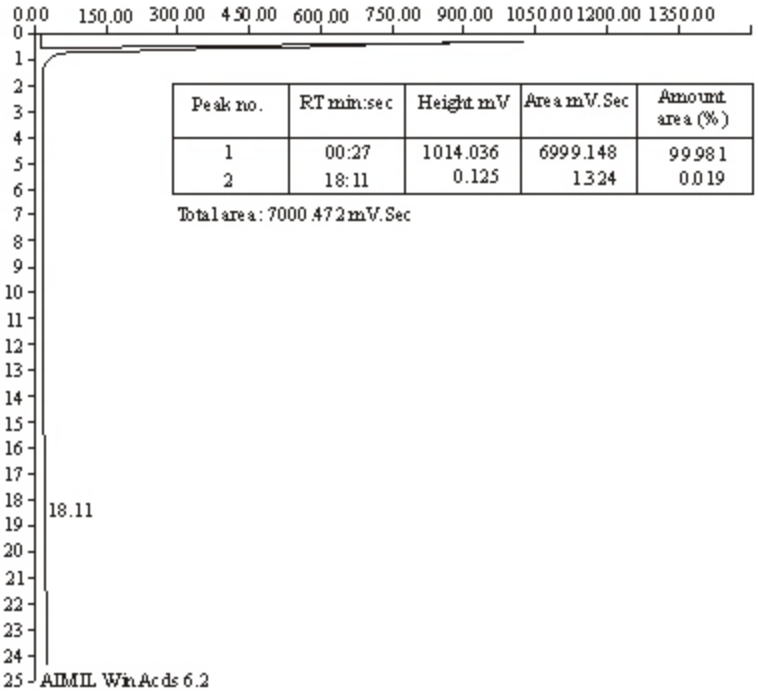

Fig. 4: Gas Chromatography analysis using Ethanol A.R.

Effect of different saccharification periods of 0.3N H_2SO_4 on the ethanol yield of sago waste, inoculated with yeast isolated from molasses: Saccharification period at 120 min recorded maximum ethanol yield for sago waste treated with 0.3N H_2SO_4 (Table 5). At 60 min, liquid waste yielded ethanol yield of about 13.1% followed by solid waste of about 7.3%. The maximum yield of ethanol for liquid waste at 120 min is about 15.0% followed by solid waste 7.5%. Moreover at all saccharification periods viz., 60 and 120 min, the ethanol was found to increase upto 15 days and after that a gradual reduction in ethanol yields was observed. Among the varying saccharification periods with 0.3N H_2SO_4, the ethanol yield increased linearly with the time of incubation upto 15 days and reduced afterwards.

In the present study, the sago wastes were acid hydrolyzed with HCl and H_2SO_4 at different concentrations and time exposures and the reducing sugar content was estimated. With the exposure of 120 min, maximum reducing sugar was obtained from the sago

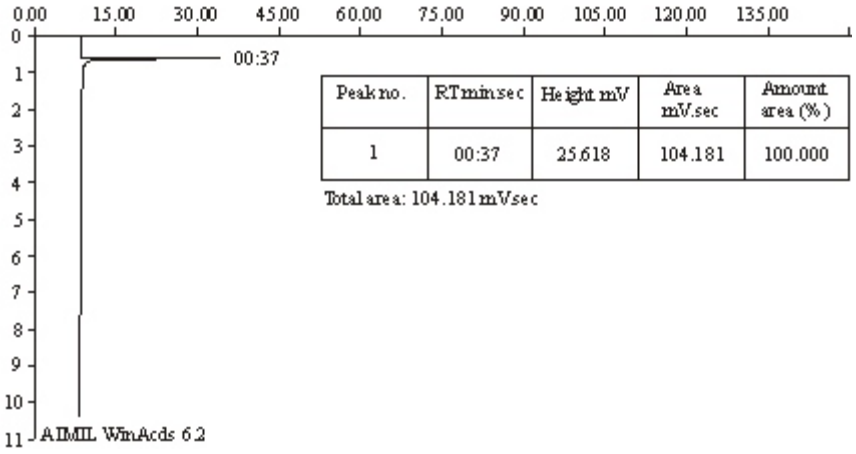

Fig. 5: Gas chromatography analysis using 0.3 N HCl at 120 min

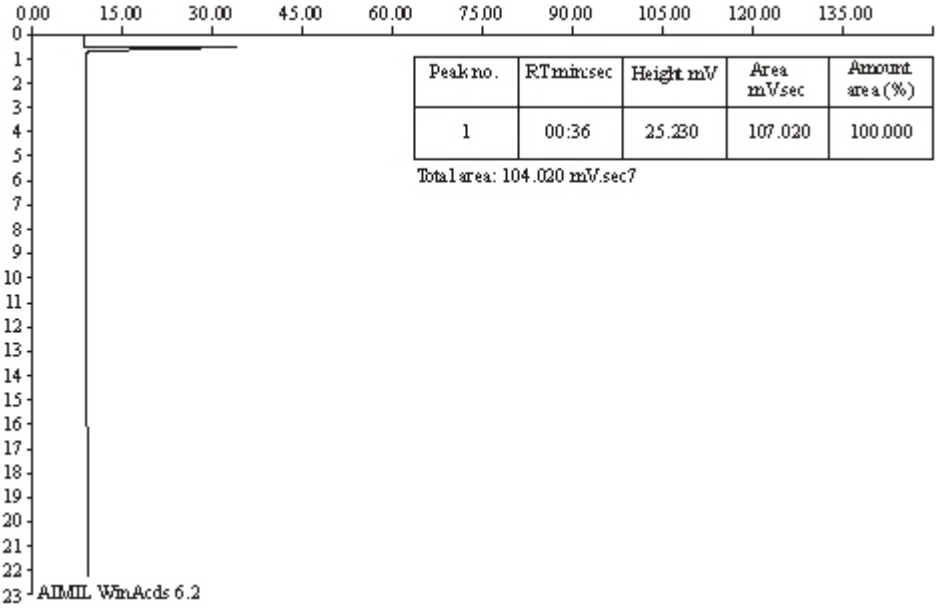

Fig. 6: Gas chromatography analysis using 0.3 N H_2SO_4 at 120 min

waste saccharified with 0.3 N HCl and H_2SO_4. The sago liquid wastes gave more amount of reducing sugar than solid waste.

Angela and Koehler (2000) viewed that conversion efficiency is increased with increase in acid concentration and the concentrated acids have more potential to break down the cellulose material or the starch materials into subunits of sugars. These 6-carbon sugars were easily utilized by the yeast and converted it into ethanol. It can be seen that out of two commercial acids used as saccharifying agents hydrochloric acid was found to be more effective for higher recovery of reducing sugars than sulphuric acid.

Gas chromatography: The ethanol was distilled using distillation unit and estimated by gas chromatography (Hari Krishna *et al*., 2000). The ethanol obtained was compared with the standard analytical grade ethanol (Fig. 4, 5 and 6). The retention time for standard analytical grade ethanol was about 27 sec, whereas fermented product using 0.3 N HCl and 0.3 N H_2SO_4 at 120 min were about 36 and 37 sec, with 100% purity.

CONCLUSION

The morphological and physiological characters of the isolates showed that the colonies of the isolates appeared butyrous, raised, smooth and glossy, utilized various sugars namely glucose, sucrose, maltose, galactose and the isolates failed to utilize lactose. Growth of yeast isolates at different level of sugar and ethanol concentration, temperature and pH revealed that, yeast

isolated from molasses recorded maximum population and it is compared with standard MTCC 173 *Saccharomyces cerevisiae*. Among the liquid and solid waste fermented, yield of ethanol was found to be more in liquid waste than the solid waste sample. The present study revealed that yeast isolated from molasses performs best on the yield of ethanol from sago liquid waste after saccharification with 0.3 N HCl and 0.3 N H_2SO_4 at 120 min than the batter isolate.

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