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Isolation of Yeasts from Raisins and Palm-Juice and Ethanol Production in Molasses Medium

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Abstract

Background/Objectives: The alternative fuels are expected to satisfy the progressive demand for energy on the wake of the negative effects of fossil fuel on the atmosphere and resultant universal warming. In this study two ethanol fermenting *Saccharomyces cerevisae* were isolated from Palm juice and Raisins. **Method/Statistical Analysis**: Both isolates were grown in Yeast extract Peptone Dextrose (YEPD) medium and characterized for alcoholic fermentation using molasses medium and optimized for pH, thermo-, osmo-, ethanol tolerance and sugar concentration. **Findings:** Results showed for ethanol fermentation, 31°C temperature, 6.01 pH and 6.50% sugar concentration is the prime condition. Raisin-isolate emerged as highly thermophilic and stress tolerant in nature. Under optimized conditions, *S. cerevisae* isolated from Palm-juice produced 9.85% of ethanol in the medium. **Application/Improvements:** Creation of ethanol through fermentation appears to be a potential other fossil fuel and can be used as exclusive fuel in vehicles with dedicated engines or in fuel blends.

Keywords: Ethanol, Molasses, Palm-Juice, Raisins, YEPD

1. Introduction

Over the last few decades, the negative effects of fossil fuel on the atmosphere and resultant universal warming, progressive demands for energy's, certain diminution of the world's energy provision, and the unsteady oil bazaar (such as the 1970s energy crisis) have improved the importance in searching for alternate fuels[1,2]. The alternative fuels are expected to satisfy several requirements including substantial greenhouse gas emission decrease, worldwide availability of raw materials, and capability of being produced from renewable feedstocks[3]. Creation of fuel ethanol through fermentation appears to be a potential alternate to fossil fuel and can be used as a only fuel in vehicles with devoted apparatuses or in fuel mixtures. Ethanol is presently derived from sugars, starches and cellulosic materials. Unconventional cellulosic materials as sea grass and potato flour also produced ethanol in previous studies[4,5]. The major two categories of raw materials are currently the core assets for ethanol creation, but sequent growth in demand for human feed related to energy could create them potentially fewer reasonable and perhaps valuable feedstock in the more near future, leave-taking the cellulosic resources as the only possible feedstock for creation of ethanol[6].

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In this learning, yeasts were separated from Palm juice as well as Raisins and fermentation was carried out in molasses media and the characteristics of the yeast isolates in terms of ethanol production, temperature and pH tolerance were also analyzed.

2. Materials and Methods

2.1 Isolation of Yeast

Raisins were collected from various fruit-shops in Dhaka and Palm juices from Tongi, Gazipur. First the sample was grown for 48 hour in YPD liquid broth and then streaked on solid YPD agar medium. Suspensions from colonies were microscopically observed to get the desired yeasts. Biochemical tests then applied to identify the ethanol-productive Yeast isolates.

2.2 Yeast Isolates Identification

The isolates were characterized by their physical characteristics e.g. colony shapes, colorant, elevation, edge and surface appearance. After that, structural and biochemical characterization were performed[7].

2.3 Maintenance of the Culture

Yeast Maintenance Medium (YMM) and Yeast extract Peptone Dextrose medium (YPD) were used to culture and maintain the Yeast isolates.

2.4 Inoculum Development

The *Saccharomyces cerevisiae* inoculums were prepared by transferring 2 to 3 visually appeared thick colonies in YPD liquid broth and growing them in 250 ml flask containing 25 ml media at 30°C and at 130 rpm for 48 hours.

2.5 Cell Count and Maintenance

A Neubauer hemocytometer was applied to count the yeast cells in each conical flask.A1 ml inoculum broth sample was serially diluted with a sterilized saline solution (0.89% w/v NaCl) to achieve the cell counting condition[8]. Most of the time the cell count recorded was around 10^6 cells/ml.

2.6 Pretreatment of Molasses

The fermentation media molasses were collected from local market in Dhaka and used as nutrient source for the growth and fermentation of yeasts. Pretreatments are performed with diluted concentration of conc. H_2SO_4 to kill unwanted micro-organisms and urea was used as nitrogen source. 250 gm molasses was diluted with tap water to measure the final volume to 1 L. And 0.10 gm urea and 0.30 ml conc. Sulfuric acid were added in media and then boiled and kept standing for 2-3 hours before use.

2.7 Fermentation

Autoclaved 10 ml molasses fermentation liquid media was inoculated with 48 hour-grown slant culture of *S. cerevisiae* and placed in a rotary incubator at 30°C in (180 rpm) vigorous shaking condition to form a homogeneous suspension.

Fermentation performed in Erlenmeyer conical flaks. 250 ml media was taken into 500 ml Erlenmeyer flasks and under aseptic condition inoculated with homogenous yeast suspension. The flasks were incubated at different temperatures in both (non-shaking and shaking) condition.

2.8 Production of Ethanol from Fermentation of Molasses

250 ml of sterile pretreated liquid fermentation media was taken into 500 ml Erlenmeyer flasks and 1000 µl of 24 hour-old culture (10^8 CFU ML^{-1}) was added and incubated. The fermentation was carried out at variable physical parameters (temperature, pH, reducing sugar concentration and agitation).

3. Stress Tolerances

3.1 Thermotolerance

One of the most important factors that affect ethanol fermentation by yeast using molasses as a carbon source is temperature. The procedure is always associated with heat-genesis that raises the temperature of the fermenter. YPD liquid broth was used for detecting thermo-tolerance and growth in liquid media of selected yeast isolates. 10 ml portion of the medium was taken into McCartney tubes, and then inoculated with two days old selected yeast isolates. The initial optical density of every tube was registered at 600 nm on spectrophotometer counter to the medium as control. Wholly cultures were hatched at

25°C, 30°C, 37°C, 40°C and 44°C for 2 days for observing thermo-tolerance of isolates. As evidence of growth increase in optical density in a tube was recorded. Besides this, growth on YPD agar media at 25°C, 30°C, 37°C and 40°C was also observed to ensure thermo-tolerance.

3.2 Ethanol Tolerance

1(One) ml of different concentrations of absolute ethanol varying from 5.0 to 25.0% (v/v) prepared, and added to altered flasks of the similar medium to create changing percentages of ethanol i.e. 5.0% (v/v) (5%, 10%, 12%, 15%, 18% 20% and 25%). 40 ml portion of the medium was taken into 125.0 ml flacks, as well as then inoculated with a selection of thermo-tolerant *Saccharomyces cerevisiae*. At 600 nm, the initial optical density of each flask was recorded on spectrophotometer counter to the medium as control. For 5 days all cultures were incubated at 40°C. As growth evidence the optical density increase (or decrease) in a flask was monitored and recorded. The ethanol tolerance of yeasts was assessed as the concentration of ethanol at which the growth of yeasts was just inhibited.

3.3 pH Tolerance

YPD liquid broth was used for detecting the ability of yeast isolate to grow in different pH. Sterilized YPD broth of various pH was prepared. Every test tube contained 13 ml of YPD media with different pH. Then every tube was inoculated by half loop full of yeasts as well as preliminary optical density was measured at 600 nm as well as incubated at 30°C for 48 hrs. Cell density was more measured at 600 nm for growth after 48 hours. Blank media was used as a control.

3.4 Osmotolerance

Under conditions of high osmotic strength growth failure is often associated with defects in the cell membrane or cytoskeletoncomponents[9]. YPD media was prepared containing 6%, 9%, 12%, 15%, 18% and 20% of NaCl. Every test-tube contained 13 ml of YPD broth with appropriate concentration of salt as well as blank medium was used as a control. Every test tube was inoculated with half loop full of, *S. cerevisae* cell as well as measurement primary optical density was made at 600 nm as well as the cells incubated at 30°C for 48 hours. Cell density was further measured at 600 nm after 2 days.

4. Estimation of Reducing Sugars

Dinitrosalicylic acid (DNS) method was applied for the estimation of the reducing sugar content in fermentation medium[10]. A double beam UV/VIS-scanning spectrophotometer was used for recording absorbance at 540 nm. In future, microwave method can be a good candidate to become alternative to IR method, chemical analysis and optical methods[11].

4.1 Estimation of Ethanol: Conway Method

1 (One) ml fermentation solution was diluted to in 250, 500 and 1000 times with purified water and 1 ml of the dilute solution was taken as a sample. A Conway unit was used for alcohol detection by the following technique.

1 ml potassium dichromate was placed into the Conway unit center as well as sample was placed round the center. The unit was then covered by a glass plate for 24 hours for reaction. The ethanol and water slowly evaporated, came in contact with potassium dichromate as well as oxidized. In one Conway unit 1 ml distilled water (around center) was used as sample, hence the unit was given status of control. After allowing for reaction for 24 hours the sample was titrated against 0.1 N Na_2SO_3.

5. Results and Discussion

5.1 Yeast Isolates Identification

The selected isolates were found to be the member of *Saccharomyces sp.,* based on white, cream-like texture of the colony characteristic, ovoid microscopic shape, the presence of ascospore and multipolar budding pattern, biochemical carbohydrate test.

5.2 Fermentation of Carbohydrates

Saccharomyces cerevisiae showed variation in sugar utilization in the study (Table 1). Seven different sugars were used for biochemical characterization test. The palm juice isolate metabolized glucose, sucrose, fructose, lactose, maltose and trehalose but failed to grow on xylose. The isolate obtained from Raisins metabolized glucose, sucrose, fructose, lactose and trehalose but failed to grow on maltose as well as xylose.

5.2.1 Stress Tolerance of Isolated Yeasts

Thermotolerance: Five YEPD Agar containing plates were streaked by yeast isolates and incubated for 48 hours at 25°C, 30°C, 37°C, 40°C and 44°C. Palm-juice isolate showed the ability to grow at 25°C-44°C, but at 44°C the Raisin isolate failed to grow. The thermotolerance study results, obtained from solid media, were confirmed by the liquid media study (Table 2).

From the given results it is evident that the Raisin isolate was slightly thermophilic as it had ability to grow up to 44°C though the most suitable condition for growth yeast found to be 30°C.

Ethanol Tolerance: The ethanol tolerance was screened by the two isolates (Palm-Juice and Raisin). The isolate showed growth curve in 20% ethanol containing liquid YEPD media. Palm-Juice isolate showed optimum growth rate at 5% ethanol containing media and Raisin isolates showed maximum in 10% ethanol. O.D of growth-curve were recorded at 5%, 10%, 12%, 15%, 18%, 20%, and 25% of ethanol containing liquid media (Table 3). In previous study, *S. italicus* exhibits exponential increase in glycogen content as a protective measure against the stress created across the plasma membrane by the action of ethanol, while grown in the presence of 2-8% v/v ethanol. But hydrophobic interactions are reduced by ethanol concentration above 8%v/v thereby depleting the insoluble glycogen content with increase in membrane permeability[12]:

pH Tolerance: The yeast isolates showed growth ability at a wide Range of pH. At high acidic pH2 the separates demonstrated the ability to grow, which was intriguing. Both isolates withstood the high alkaline condition up to pH 10. Optimum growth was recorded at pH 5. Cell density was recorded at 600 nm (Table 4), after 48 hours. The pH range varied from pH 4.0 to 6.0 for optimum growth of yeast. And also dependency on temperature, the oxygen-presence, and the nature of yeast strain was observed. Narendranath & Power[13] reported similar observation for pH tolerance of yeasts.

Osmotolerance: 6%, 9%, 12%, 15%, 18%, and 20% of NaCl containing YEPD liquid media was prepared. At 6%, 9%, 12%, 15%, 18%, and 20% of salt containing media O.D of growth pattern was recorded (Table 5):

Osmoltolerant characteristic of both isolates were observed by the resistance to high concentration of NaCl. Both isolates had their highest growth at 6% NaCl

Table 1. Fermentation of different carbohydrates by selected Palm-juice isolated strain of S. cerevisiae.

Carbohydrate	Before Fermentation	After Fermentation
Glucose/ Dextrose	Reddish or Pink	+ (yellow), gas
Sucrose	Reddish or Pink	+ (yellow), gas
Maltose	Reddish or Pink	+ (yellow), gas
Lactose	Reddish or Pink	+ (yellow), gas
Fructose	Reddish or Pink	+ (yellow), gas
Xylose	Reddish or Pink	- (no color change)
Trehalose	Reddish or Pink	+(yellow), gas formed

Table 2. Growth of PJ and R isolate at different temperature in liquid media.

Temperature	Strain	O.D. at Inoculation	O.D. after 24 hours	O.D. after 48 hours
25°	palm juice	0.57	1.61	2.79
	Raisins	0.53	1.54	2.36
30°	palm juice	0.52	1.86	2.51
	Raisins	0.47	1.83	2.36
37°	palm juice	0.71	1.95	2.16
	Raisins	0.55	1.13	1.97
40°	palm juice	0.45	1.36	1.99
	Raisins	0.37	0.60	1.29
44°	palm juice	0.56	0.39	0.82
	Raisins	0.65	0.63	0.57

Table 3. Growth of PJ and R isolates in different concentration of ethanol in the media.

Ethanol %	Isolate	O.D. at Inoculation	O.D. after 24 hours	O.D. after 48 hours
5	Palm juice	0.38	0.76	1.9
	Raisins	0.49	0.99	1.4
10	Palm juice	0.36	1.36	1.8
	Raisins	0.55	0.70	1.5
12	Palm juice	0.27	0.70	1.2
	Raisins	0.35	0.40	0.6
15	Palm juice	0.36	0.69	0.9
	Raisins	0.26	0.30	0.3
18	Palm juice	0.30	0.68	1.3
	Raisins	0.28	0.30	0.3
20	Palm juice	0.31	0.39	0.4
	Raisins	0.21	0.30	0.5
25	Palm juice	0.26	0.26	0.1
	Raisins	0.30	0.28	0.1

Table 4. Growth of selected Yeast isolates in liquid media at different pH.

pH	Isolate	O.D. at Inoculation	O.D. after 24 hours	O.D. after 48 hours
2	Palm-juice	0.39	0.58	0.79
	Raisins	0.36	0.49	0.55
3	Palm-juice	0.37	1.14	1.51
	Raisins	0.45	0.86	1.50
4	Palm-juice	0.45	1.60	1.96
	Raisins	0.39	0.81	1.57
5	Palm-juice	0.44	1.47	1.99
	Raisins	0.40	0.89	1.67
6	Palm-juice	0.56	0.59	1.90
	Raisins	0.48	0.97	1.67
7	Palm-juice	0.48	1.17	1.86
	Raisins	0.45	0.75	1.38
8	Palm-juice	0.40	0.86	1.64
	Raisins	0.35	0.67	1.14
9	Palm-juice	0.39	0.65	1.54
	Raisins	0.36	0.84	1.30
10	Palm-juice	0.48	0.79	1.28
	Raisins	0.39	0.75	1.38

Table 5. Growth in different concentration of NaCl in the liquid media.

% of NaCl	Isolate	O.D. at Inoculation	O.D. after 24 hours	O.D. after 48 hours
6	Palm-juice	0.22	0.58	1.37
	Raisins	0.23	0.35	0.44
9	Palm-juice	0.24	0.39	0.69
	Raisins	0.25	0.26	0.34
12	Palm-juice	0.23	0.26	0.35
	Raisins	0.23	0.26	0.31
15	Palm-juice	0.26	0.23	0.32
	Raisins	0.29	0.26	0.20
18	Palm-juice	0.26	0.29	0.34
	Raisins	0.24	0.29	0.36
20	Palm-juice	0.38	0.34	0.37
	Raisins	0.26	0.25	0.35

containing media and tolerated up to 12% NaCl in the medium and gradually declined (Table 5).

5.2.2 Kinetics of Ethanol Fermentation by the Isolates under Optimum Growth Condition

S. cerevisiae is capable of very fast glycolysis and ethanol production under optimized condition. The Palm juice isolates were grown in molasses fermentation media under optimum sugar concentration, temperature and two different pH and the results are shown in Figures 1 and 2. The production of ethanol was zero at the initial stage of fermentation where the pH was fixed at 5 (Figure 1). After 24 hours, the level of glucose dwindled and significant amount of ethanol was produced. 48 to 72 hour's fermentation resulted the maximum production of ethanol. The pH changes positively in 48 hours, but then slightly decreased. Earlier experiments suggested shaking is more favorable than non-shaking state to produce ethanol. After 96 hours of shaking fermentation maximum 9.85% Ethanol production observed.

In pH 5 the glucose diminished rapidly and ethanol production rate was not faster (Figure 2). pH change was similar to the previous fermentation. Both process showed the tendency of adjusting the pH at the same level after 24 hours.

Glucose concentration is pivotal in ethanol fermentation. Various set of parameters showed production of ethanol is favorable in 6.5% glucose.

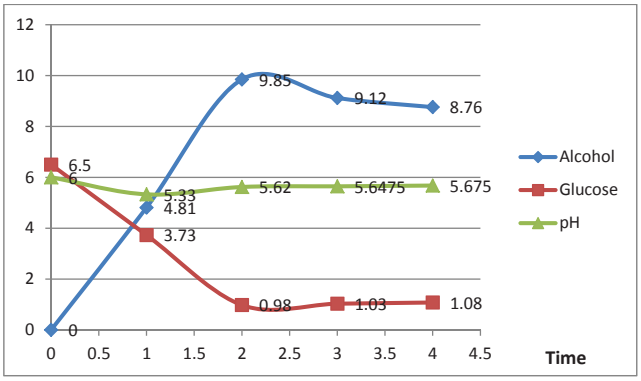

Figure 1. Fermentation Kinetics of Palm-juice yeast strain in shake flasks at 6.5% reducing sugar and pH 6.0 at 30° temperature.

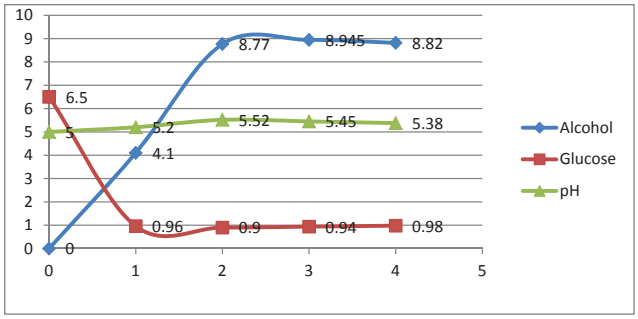

Figure 2. Fermentation Kinetics of Palm-juice yeast strain in shake flasks at 6.5% reducing sugar and pH 5.0 at 30° temperature.

In a similar study in Bangladesh, five isolates were reported to produce ethanol by fermenting molasses at wide range of temperature (25-37°C). The production was maximum at 30°C after 48 hours of incubation. Using glucose as substrate in the fermented media varied between 2.3%-5.90%, the alcohol production rate were reported to be maximum up to 36 hours[14].

The logarithmic relationship between time of fermentation and initial concentrations of sugar was demonstrated in previous fermentation study with various initial concentrations of sugar[15,16].

The alcohol production in the fermentation broth was increased with the 5% (v/v) glucose but plummeted beyond that in another similar experiment. In the fermentation broth the final glucose utilization was found to be used up at the glucose concentration equal to or below 5% (v/v), but above the glucose concentration of 5% (v/v), the final glucose utilization is remarkable. The maximum specific growth rate and maximum ethanol concentration were increasing with an increase of glucose concentration for 5% (v/v). When glucose concentration was greater than 5% (v/v) ethanol production reduced and cell growth were dwindled[17]. Intriguingly, similar findings were observed in this experiment.

6.5% and 7% glucose concentration showed to be the optimum sugar concentration for Ethanol production. In 7.5% concentration production plummeted. Palm-juice isolates seems to be the most productive isolate than the Raisins isolates.

Because of importance in regulatingmicrobial contamination as its influence on cell growth, fermentation rates and by-product formation hydrogen ion concentration has a significant impact on industrial fermentation. The optimum alcohol yields are generally obtained at pH 4.5-4.7. More glycerol and organic acids are formed at the expense of ethanol at higher pH[18]. pH 6.0 proved to be more suitable condition than pH 5.0 for the production of ethanol in this experiment.

6. Conclusion

Six out of seven sugars (Glucose, Sucrose, Fructose, Lactose, Maltose and Trehalose) were successfully fermented by the Palm-juice yeast isolates. The isolates failed to ferment Xylose. And except Maltose and Xylose five out of seven sugars were fermented by the isolates from Raisins. This biochemical characterization test proved the identity of both microorganisms as *Saccharomyces cerevisiae.*

Both isolates were screened for alcohol tolerance as well as showed up to 25% ethanol tolerance in YEPD liquid media. Atardy growth rate was observed at 10-20% ethanol containing media.

Depending on temperature, the presence of O_2, and the strain of yeast, the optimal pH range for growth of *Saccharomyces cerevisiae*can vary from pH 4.0 to 6.0. This is likely due to the optimum pH value for the activity of plasma membrane-bound proteins, including various enzymes and transport proteins[13]. In our study the is Palm-juice can grow in a wide pH range from 2 to 10, but ph 5.0 showed to be the optimum pH for it. The Raisins strain showed growth in pH 3 to 10, but in pH 2, it grows reluctantly. One of the most important findings of this experiment is the ability of growing in high acidic condition by the Palm-juice isolate.

The results proved both isolates have resistance against higher osmotic pressure. Both strains showed their highest growth in 6% NaCl containing media and growth gradually decreased in 15% and 20% NaCl containing media (Table 5).

A series of experiments had been conducted at different glucose concentration to determine the optimum condition for twenty-four hours alcoholic fermentation. In shaking condition, the Palm-juice strain showed highest 6.93% production in 6.5% and 7% glucose containing medium. In the same parameters the Raisins strain produced 6.93% in 6% glucose concentration. In the non-shaking condition, the Palm-juice strain showed 5.53% production in 4% glucose level and the Raisins resulted maximum 4.1% in the same glucose concentration.

The seventy-two hours fermentation results showed the detailed characterization of the Palm-juice strain, which proves to be the better strain in every aspect. Maximum 9.85% alcohol production was recorded after forty-eight hours in 6.5% glucose and pH 6 (Figure 1). This proved to be the highest production achieved in molasses media in this experiment.

7. References

1. Himmel ME, et al. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. Science. 2007; 315(5813):804-07.
2. Solomon BD, et al. Grain and cellulosic ethanol: History, economics, and energy policy. Biomass & Bioenergy, 2007; 31(6):416-25.

3. Hagerdal HB, Galbe M, Grauslund GMF, Liden G and Zacchi G. Bio-ethanol - the Fuel of Tomorrow from the Residues of Today. Trends in Biotechnology. 2006; 24(12):549-56.
4. Ravikumar S, Gokulakrishnan R, Kanagavel M, Thajuddin N. Production of Biofuel Ethanol from Pretreated Seagrass by Using Saccharomyces cerevisiae. Indian Journal of Science and Technology. 2011 Sep; 4(9). doi:10.17485/ijst/2011/v4i9/30235.
5. Rani P, Sharma S, Garg FC, Raj Kushal, Wati Leela. Ethanol Production from Potato Flour by Saccharomyces cerevisiae. Indian Journal of Science and Technology. 2010 July; 3(7). doi:10.17485/ijst/2010/v3i7/29804.
6. Taherzadeh MJ and Karimi K. Process for ethanol from lignocellulosic materials I: Acid-based hydrolysis processes. BioResources. 2007; 2(3):472-99.
7. Boboye B, Dayo-Owoyemi I. Evaluation of dough sensory properties impacted by yeasts isolated form cassava. J. Applied Sci. 2009; 9(4):771-76.
8. Alfenore S, Molina-Jouve C, Guillouet SE, Uribelarrea JL, Goma G and Benbadis L. Improving ethanol production and viability of Saccharomyces cerevisiae by a vitamin feeding strategy during fed batch process. Appl. Microbiol. Biotechnol. 2002 Oct; 60(1):67-72.
9. Novick P and Botstein D. Phenotypic analysis of temperature-sensitive yeast act in mutants. Cell. 1985 Feb; 40(2): 405-16.
10. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1985; 31(3):426-28.
11. Beulah Jackson, T. Jayanthy. Determination of Sucrose in Raw Sugarcane Juice by Microwave Method. Indian Journal of Science and Technology. 2014 Jan; 7(5). doi:10.17485/ijst/2014/v7i5/49725.
12. Dake MS, Khetmalas MB, Amarapurkar SV. Role of Insoluble Glycogen in Ethanol Adaptation Mechanism of Saccharomyces italicus. Indian Journal of Science and Technology. 2011 Jan; 4(1). doi:10.17485/ijst/2011/v4i1/29932.
13. Narendranath NV and Power R. Relationship between pH and medium dissolved solids in terms of growth and metabolism of Lactobacilli and Saccharomyces cerevisiae during ethanol production. Appl. Env. Microbiol. 2005; 71(5):2239-43.
14. Khan AR, Malek MA, Choudhury N and Khan SI. Alcohol production from molasses and liquid sugar using some indigenous yeast isolates. Bangladesh Journal of Microbiology. 1989; 6(1):37-42.
15. Borzani W, Garab A, Pires MH, Piplovic R and De IaHiguera GA. Batch ethanol fermentation of molasses: a correlation between the time necessary to complete the fermentation and the initial concentrations of sugar and yeast cells. World J Microbiol Biotechnol. 1993; 9(2):265-68.
16. Bertolini MC, Ernandes JR and Laluce C. New yeast strains for alcoholic fermentation at higher sugar concentration. Biotechnol Lett. 1991; 13(3):197-202.
17. Sripiromrak A. Isolation and characterization of thermotolerant yeast for ethanol production, Suranaree University of Technology. 2006
18. Wayman M and Parekh SR. Microbiology of fermentation catalysts. Milton Keynes: Open University Press: Biotechnology of Biomass Conversion. 1990; p. 75-100.