International Journal of Renewable and Sustainable Energy
2013; 2(4): 133-139
Published online July 10, 2013 (http://www.sciencepublishinggroup.com/j/ijrse)
doi: 10.11648/j.ijrse.20130204.11

Process optimization of bioethanol production by stress tolerant yeasts isolated from agro-industrial waste

Md. Fakruddin[1], Md. Ariful Islam[2], Monzur Morshed Ahmed[1, *], Nayuum Chowdhury[2]

[1]Industrial Microbiology Laboratory, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial research (BCSIR), Dhaka, Bangladesh
[2]Departments of Biotechnology, BRAC University, Dhaka, Bangladesh

E mail address:
monzur_29@yahoo.com(M. M. Ahmed)

To cite this article:
Md. Fakruddin, Md. Ariful Islam, Monzur Morshed Ahmed, Nayuum Chowdhury. Process Optimization of Bioethanol Production by Stress Tolerant Yeasts Isolated From Agro-Industrial Waste. *International Journal of Renewable and Sustainable Energy.* Vol. 2, No. 4, 2013, pp. 133-139. doi: 10.11648/j.ijrse.20130204.11

Abstract: The need of Bioethanol or biofuel is increasing worldwide day by day due to renewable resources and ease of production form cheap raw materials. There are several factors that affect on bioethanol production by using yeast. The main objective of this research work was to isolate stress tolerant yeast from agro industry and optimize a process for ethanol production by considering all the factors. Several fermentation batches were carried out by 3 stress tolerant strains varying temperature, pH, sugar concentration, aeration, immobilization and metal ions. From different experiments it was found that temperature 30°C, reducing sugar concentration ranged between 5-6%, pH between 5.0 - 6.0 and shaking condition were optimum for maximum yield of ethanol by strains *Saccharomyces unisporous* (P), *Saccharomyces cerevisiae* (C) and (T).The Bioethanol production capacity of yeasts were found P -15.00%, C -12.50% and T – 10.15%at pH 6.0, 30°C temperature in media with 5.5% initial reducing sugar concentration in shaking condition (115 rpm).Pilot scale ethanol production by P strain was 13.10%, C strain 11.15% andT strain 9.80% at 60 hours. Immobilized cells were produced more ethanol than free cells with same culture conditions. Effect of potassium, magnesium, chromium and boron was investigated on ethanol production. Potassium, Magnesium was shown stimulatory effect on ethanol production.

Keywords: Ethanol, Molassesand Stress Tolerant

1. Introduction

Bioethanol is being widely investigated as a renewable fuel source because in many respects it is superior to gasoline fuel [1]. Ethanol provides energy that is renewable and less carbon intensive than oil. Several studies have shown that sugarcane-based ethanol reduces greenhouse gases by 86 to 90% [2, 3]. The most well-known and commercially significant yeasts that been primarily used for bioethanol production are the related species and strains of *Saccharomyces cerevisiae* [4]. These organisms have long been utilized to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages and in the backing industry [5]. One yeast cell can ferment approximately its own weight of glucose per hour. Sugars from sugar cane, sugar beets, molasses, and fruits can be converted to ethanol directly. The most widely used sugar for ethanol fermentation is blackstrap molasses which contains about 35 – 40 wt% sucrose, 15 – 20 wt% invert sugars such as glucose and fructose, and 28 – 35 wt% of non-sugar solids [6]. Generally, to obtain high quality and yield of ethanol in ethanol industry, selection of fermentative yeast is very essential. Tolerance to high temperatures and high ethanol concentrations are important properties of microorganisms of interest to industry [7]. The ability of yeast to produce ethanol depends on many factors such as strains, growth factors and optimum environmental conditions [8].

The aim of this study was to optimize process parameters of bioethanol production by stress-tolerant yeasts isolated from agro-industrial waste of Bangladesh.

2. Material and Methods

2.1. Collection of Strain

The yeast strains were isolated from agro industries of Bangladesh. The strains were coded as P, C and T.

2.2. Identification of Yeast Isolates

The yeast strains were characterized based on their cultural characteristics (Colony shapes, pigment, elevation, edge and surface appearance). Morphological and biochemical characterization of the isolated yeasts was performed according to Boboye and Dayo-Owoyemi [9].

2.3. Maintenance of the Culture

The Yeast strains were cultured and maintained in Yeast Maintenance Medium (YMM) and Yeas extract Peptone Dextrose medium (YPD).

2.4. Molasses Pretreatment

The molasses were collected from local market and used as nutrient source for the Yeast. Molasses were pretreated with sulfuric acid to remove particles, dirt and kill unwanted microbes and Urea was used as nitrogen source. 250 gm molasses is diluted with 1 L water and 0.10 gm urea and 30 ml conc. Sulfuric acid were added. It was then heated to the boiling and kept standing for couple of hours before use [10].

2.5. Reducing Sugar Estimation

The concentration of reducing sugar of fermentation media was measured by DNS method [11].

2.6. Ethanol Production Procedure from Fermentation of Molasses

250 ml of sterile pretreated fermentation media was taken into 500ml Erlenmeyer flasks and then added 1000 µl of 24 h culture (10^8 CFU ML^{-1}) and incubated for certain time with different condition. The fermentation was carried out at varying temperature, pH, reducing sugar concentration, agitation and immobilized condition.

2.7. Alcohol Estimation

Alcohol percentage in the fermentation broth was measured by redox titration (micro- difusion) method [12].

3. Results

3.1. Identification of Yeast Isolates

Based on the colony characteristics (white and creamy texture) ovoid microscope shape, the presence of ascospore and budding pattern (multipolar), the selected isolate was found to belong S*accharomyces* type unicellular ascomycete according to Lodder [13] and Boekhout and Kurtzman [14].

3.2. Effect of Temperature

Temperature is one of the most important factors that affect the ethanol production by yeast using molasses as a carbon sources. The fermentation process is always accompanied with evolution of heat that raises the temperature of the fermenter. As a result it becomes necessary to cool the large fermenters in the distilleries. All the 3 strains showed marked increased production at temperature 30°C and ethanol production at temperature 35°C was almost similar to that of 30°C but ethanol production gradually decreased at temperature 40°C (Fig.1). At 30°C ethanol production by P strain was 15% (v/v), by C strain was 12.5% (v/v) and by T strain was 10.15% (v/v). At temperature 35°C ethanol production by P strain was 10.62% (v/v), by C strain was 10.26% (v/v) and by T strain was 7.2% (v/v). Production gradually decreased at temperature 40°C as strain P produced 7.00% (v/v) ethanol, strain C produced 6.50% (v/v) and strain T produced 4.75% (v/v). Ethanol production at various temperatures indicates that 30°C is optimum for P, C and T strains.

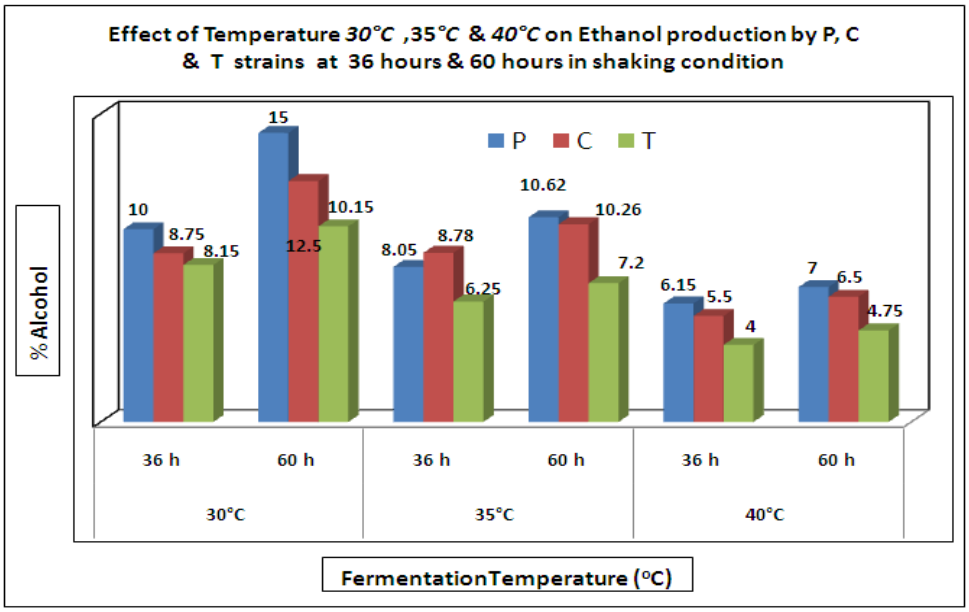

Figure 1. *Effect of fermentation temperature on ethanol production*

3.3. Effect of Reducing Sugar

It was found that all the three strains produced maximum ethanol at initial reducing sugar concentration 6% after 60 hours. Ethanol production level was decreased due to increase or decreases in the initial reducing sugar concentration from 6% (Fig.2). Ethanol production rate increases slightly when initial reducing sugar concentration increases from 5 to 6% but production decreases slightly at 8% and 10%. So the optimum reducing sugar percentage for all 3 strains (P, C and T) is within 5 to 6%.

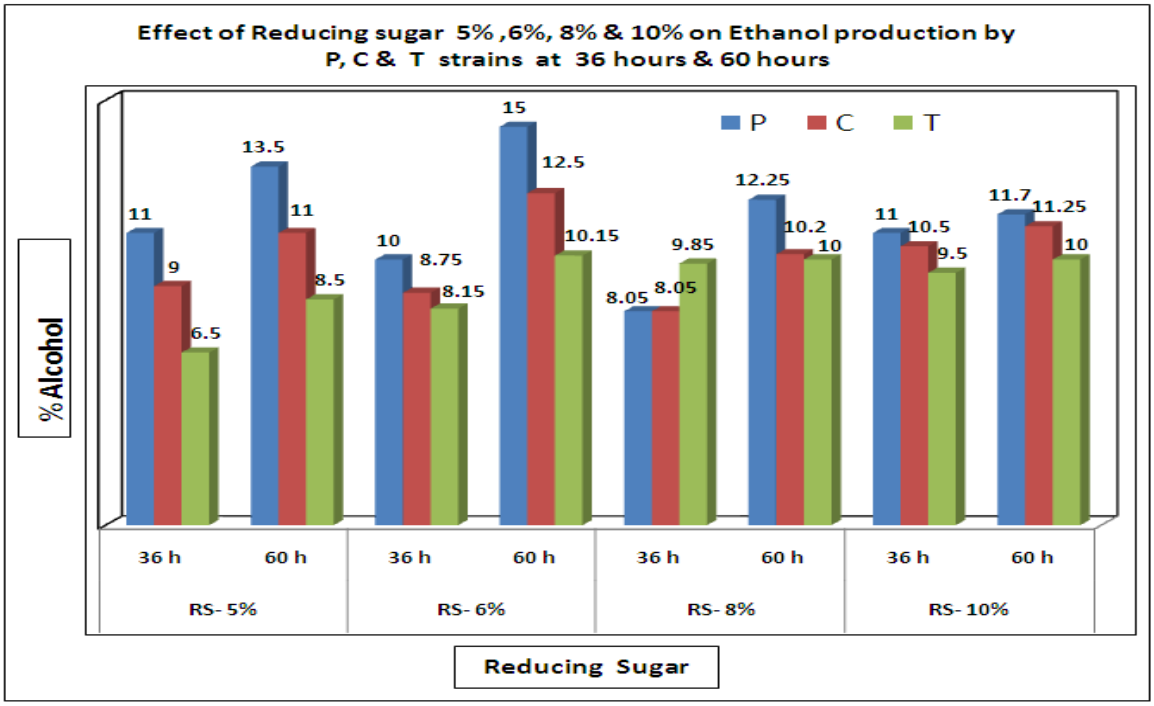

Figure 2. *Effect of reducing sugar on Ethanol Production*

3.4. Effect of PH

Initial pH (5.5) of the fermentation media had great influence on ethanol production by all strains. Increases or decreases in the initial pH from 5.5 of the fermentation media have marked decreases in the ethanol yield (Fig. 3). It was found that all the strains produced maximum ethanol at pH 5.5 after 60 hours except T strain. The maximum yield of ethanol by P strain was 15% (v/v), by C strain was 12.50% (v/v) at pH 5.5 and by T strain was 10.60% (v/v) at pH 6.00 (Fig.3).

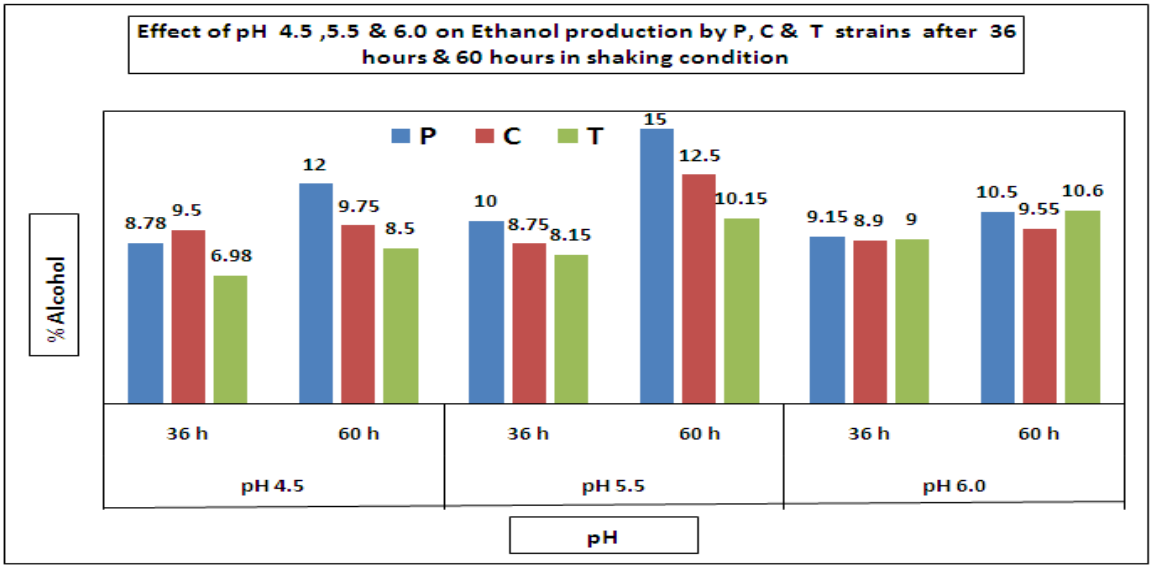

Figure 3. *Effect of pH on Ethanol Production*

3.5. Effect of Metal

To observe the effect of metals on ethanol production by the selected yeast strains, four metal salt was added in the fermentation media as a source of metal ion. The metal salts are Potassium dichromcate, magnesium chloride, copper sulphate and boric acid. Potassium dichromcate showed marked stimulatory effect, magnesium chloride showed slight stimulatory effect but copper sulphate and boric acid showed inhibitory or no effect on ethanol production (Fig.4).

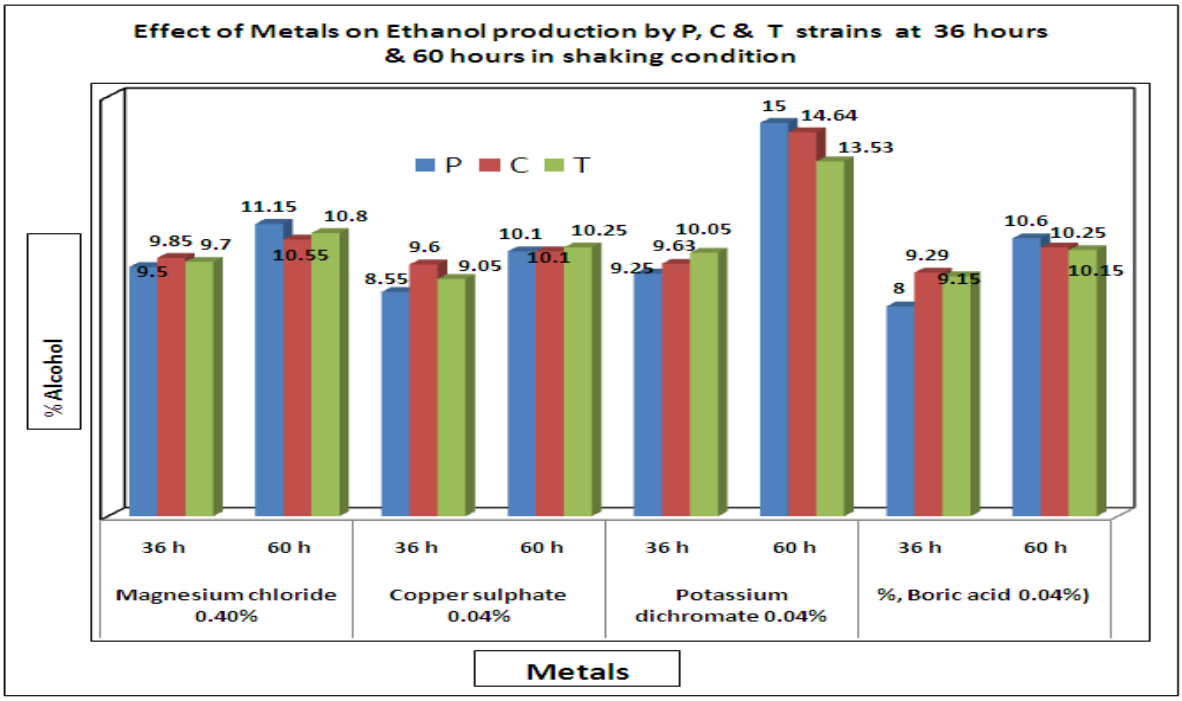

Figure 4. *Effect of metals on Ethanol Production*

3.6. Effect of Immobilization

Immobilization was done according to Mariam *et al.* [15] and it showed significant effect on ethanol yield. Immobilized cell markedly increases ethanol yield than free cell. At optimum condition temperature 30°C, pH 5.5 & initial reducing sugar concentration 6%, free cells of P strain produced 15% (v/v), C strain produced 12.5% (v/v) and T strain produced 10.15% (v/v) ethanol after 60 hours. Immobilized cells of P strain produced 15.50% (v/v), C strain produced 14.65% (v/v) and T strain produced 13.10% (v/v) (Fig. 5) at the same condition.

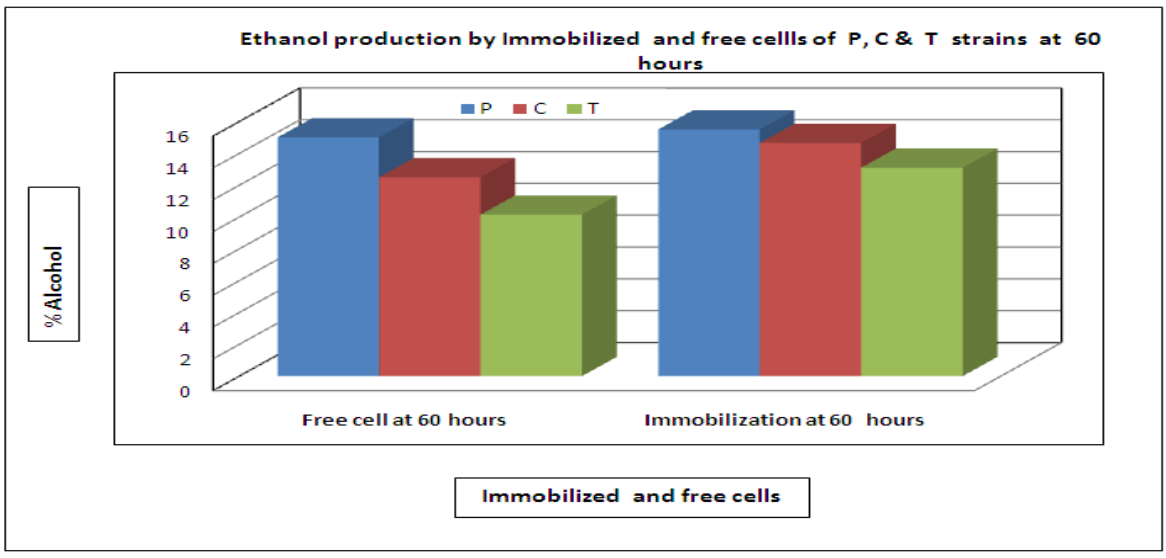

Figure 5.*Effect of Immobilization on Ethanol Production*

3.7. Pilot Scale Ethanol Production from Fermentation of Molasses

After successful production of ethanol at lab scale it is very important to know the capability of the yeast strain to produce ethanol in pilot scale to make a strain commercially feasible. For this reason pilot scale production of the yeast strains were observed. All the strains produced almost similar percentage of ethanol to that produced at small scale (Shake flask). Al pilot scale ethanol production by P strain was 13.10%, by C strain was 11.15% and by T strain was 9.80% after 60 hours.

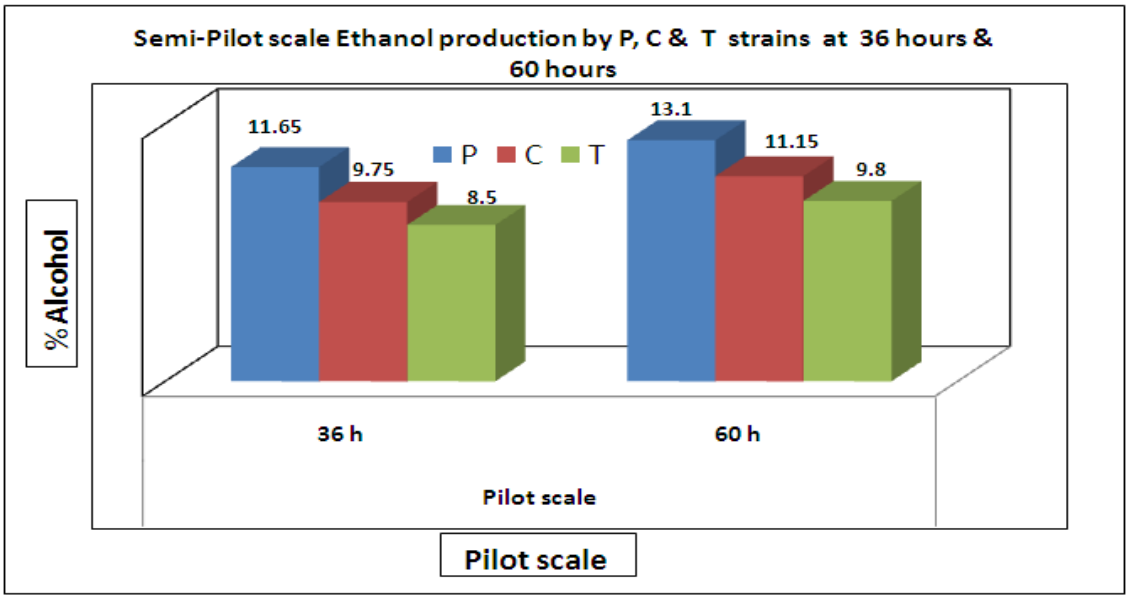

Figure 6. *Pilot scale ethanol Production of ethanol using initial reducing sugar concentration 5.50% at 30°C in shaking condition.*

4. Discussion

Samples were collected from different sources of agro industries. Based on some morphological and physiological characterization presumptive yeast isolates has been selected. Based on the colony characteristics (white and creamy texture) ovoid microscope shape, the presence of ascospore and budding pattern (multipolar), the selected isolate was found to belong *Saccharomyces* type unicellular ascomycete according to Lodder [13] and Boekhout and Kurtzman [14].

Temperature is one of the most important environmental factors affecting microbial activity. To determine optimum temperature for ethanol fermentation, the fermentation media were kept at 30, 35 and 40°C maintaining initial reducing sugar concentration 6% and pH 5.5. The ethanol yield was determined up to 60 hours. The maximum ethanol production was obtained at 30°C which was 15.00% (v/v) by P strain, 12.50% (v/v) by C strain and 10.15% (v/v) by T strain. At temperature 35°C ethanol yield slightly decreased as strain P produced 10.62% (v/v), strain C produced 10.26% (v/v) and strain T produced 7.20% (v/v) ethanol but with increasing temperature yield was drastically decreased as at temperature 40°C, strain P produced 7.00% (v/v), strain C produced 6.50% (v/v) and strain T produced 4.75% (v/v) ethanol. Ethanol yield at temperature 30°C and 35°C was almost similar. So these strains can produce ethanol at higher temperature 30°C or 35°C and will be suitable for industrial scale.

Reducing sugar is one of the important factors that may affect markedly in the cost of ethanol production. The profitability of ethanol production is dependent on favorable sugar cane molasses price and the quality of molasses (sugar %) [16]. To determine the effect of reducing sugar several experiments were conducted at different initial reducing sugar concentration with maintaining incubation temperature 30°C and pH of the medium 5.5 in shaking condition. Ethanol yield increases from 5% to 6% reducing sugar concentration but decreases slightly with increases concentration. At 5% initial reducing sugar, ethanol yield by P strain was 13.5% (v/v), by C strain was 11.00% (v/v) and by T strain was 10.15% (v/v) but at 6% initial reducing sugar ethanol yield gradually increased as P strain produced 15% (v/v), C strain produced 12.50% (v/v) and T strain produced 10.15% (v/v). At 8% and 10% reducing sugar concentration ethanol yield slightly decreased and significant amount of reducing sugar remain unutilized (2.0-2.5%).

The rate of ethanol production by yeast cells is highly affected by the pH of the fermentation medium. More acidic and basic conditions both retard the yeast metabolic pathways and hence the growth of cells [17]. So, optimum pH is required for growth and ethanol yield by the yeast strains. To determine the optimum pH for ethanol yield several experiment were conducted at pH 4.5, 5.0 and 6. Increases or decreases the initial pH from 5.5 of the

fermentation media decreases the ethanol yield (Fig. 3). All the strains are produced maximum ethanol at pH 5.5 after 60 hours except T strain. The maximum yield of ethanol by P strain was 15% (v/v), C strain was 12.50 (v/v) at pH 5.5 and T strain was 10.60 (v/v) at pH 6.00 of the fermentation medium.

Limited availability of metal ions can influence fermentation performance of yeasts. In addition, during fermentation, the concentrations of various nutrients changes and yeasts must respond dynamically to such changes [18]. To observe the effect of metals on ethanol production by yeast strains four metal salt was added in the fermentation media as a source of metal ion. The metal salts are Potassium dichromcate, magnesium chloride, copper sulphate and boric acid. Potassium dichromcate showed marked stimulatory effect, magnesium chloride showed slight stimulatory effect but copper sulphate and boric acid showed inhibitory or no effect on ethanol production. The ethanol production increased to 15.50% (v/v) for P strain, 14.64% (v/v) for C strain and 13.53% (v/v) for T strain when Potassium dichromcate was added.

Immobilized cell markedly increases ethanol yield than free cell at optimum condition. At optimum condition temperature 30°C, pH 5.5 initial reducing sugar 6%, free cells of P strain produced 15% (v/v), C strain produced 12.5% (v/v) and T strain produced 10.15% (v/v) ethanol after 60 hours whereas immobilized cells of P strain produced 15.50% (v/v), C strain produced 14.65% (v/v) and T strain produced 13.10% (v/v).

To make a strain commercially feasible it is required observing pilot scale production after successful lab scale production. For this reason pilot scale production of the yeast strains were observed. All the strains were produce almost similar to that produced at small scale (Shake flask). Al pilot scale ethanol production by P strain was 13.10%, by C strain was 11.15% and by T strain was 9.80% after 60 hours.

5. Conclusion

The fermentation of molasses using Strains P, C & T under different conditions, showed that reducing sugar concentration 5.0%- 6.0%, temperature 30°C and pH 5.0 to 6.0 are suitable for ethanol production by free cells and immobilized cells at 36 hrs and 60 hours in shaking condition. After 60 hours, ethanol production decreases due to substrate limitations or product inhibition. Immobilized cells are better in terms of ethanol production than free cells. Some metals such as Potassium dichromate and magnesium chloride etc. have stimulatory effect on ethanol production. Pilot scale production is almost similar to that observed with small scale (shake flasks 250 ml) production. These strains could be potential for ethanol production from cane molasses in commercial scale. Productivity can also be improved by mutation through radiation or genetic manipulation. Metabolic pathway engineering to direct ethanol production may a promising way to improve productivity.

References

[1] Jones, A.M., K.C. Thomas and W.M. Inglew, Ethanolic fermentation of molasses and sugarcane juice using very high gravity technology. Journal of Agricultural Chemistry, 1994. 42: p. 1242-12 46.

[2] Isaias, M., V. Leal, L.M. Ramos and J.A. Da-Silva, Assessment of greenhouse gas emissions in the production and use of fuel ethanol in Brazil.Secretariat of the Environment, Government of the State of Sao Paulo, 2004.

[3] Goettemoeller, J. and A. Goettemoeller, Sustainable Ethanol: Biofuels, Biorefineries, Cellulosic Biomass, Flex-Fuel Vehicles, and Sustainable Farming for Energy Independence. Praire Oak Publishing, Maryville, Missouri, ISBN: 9780978629304, 2007. p. 42.

[4] Chandel, A.K., E.S. Chan, R. Rudravaram, M.L. Narasu, L.V. Rao and P. Ravindra, Economics and Enviromental Impact of Bioethanol Production Technologies: an Appraisal. Biotechnology and Molecular Biology Review, 2007. 2(1): p. 14-32.

[5] Tsuyoshi, N., R. Fudou, S. Yamanaka, M. Kozaki, N. Tamang, S. Thapa, J.P. Tamang, Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation.International Journal of Food Microbiology, 2005. 9(2): p. 135-146.

[6] Osunkoya, O.A. and N.J. Okwudinka, Utilization of sugar refinery waste (molasses) for ethanol productionusing *Saccharomyces cerevisiae*.American Journal of Scientific and Industrial Research, 2011. 2(4): p. 694-706.

[7] Somda, M.K., A. Savadogo, C.A.T. Ouattara, A.S. Ouattara and A.S. Traore, Thermotolerant and Alcohol-tolerant yeasts targeted to optimize hydrolyzation from mango peel for high bioethanol production. Asian Journal of Biotechnology, 2011. 3 (1): p. 77-83.

[8] Khongsay, N., L. Laopaiboon and P. Laopaiboon, Growth and Batch fermentation of *Saccharomyces cerevisiae* on sweet sorghum stem juice under normal and very high gravity conditions. Biotechnology, 2010. 9(1): p. 9-16.

[9] Boboye, B. andI. Dayo-Owoyemi, Evaluation of Dough Sensory Properties Impacted by Yeasts Isolated from Cassava. Journal of Applied Sciences, 2009. 9(4): p. 771-776.

[10] Fakruddin, M., M.A. Quayum, M.M. Ahmed, N. Chowdhury, Analysis of Key Factors Affecting Ethanol Production by *Saccharomyces cerevisiae* IFST-072011. Biotechnology, 2012. 11(4): p. 248-252.

[11] Miller, G.L., Use of dinitrosalicyclic acid reagent for determination of reducing sugar. Analytical Chemistry, 1959. 31: p. 426-28.

[12] Conway, E.J., Microdiffusion analysis and volumetric error. Crosby Lockwood and Son, London, 1939.

[13] Lodder, J., The yeasts: A Taxonomic study. NorthHoll and Publishing, Amsterdam, 1971.

[14] Boekhout, T. and C.P. Kurtzman, Principles and methods

used in yeast classification, and an overview of currently accepted yeast genera. In Wolf, K. Nonconventional Yeasts in Biotechnology: A Handbook. Springer-Verlag: Heidelberg. 1996. p. 1-99

[15] Mariam, I., K. Manzoor, S. Ali and Ikram-Ul-Haq, Enhanced production of from free and immobilized *Saccharomyces cerevisiae*under stationary culture. Pakistan Journal of Botany, 2009. 41: p. 821823.

[16] Arshad, M., M.A. Zia, M. Asghar and H. Bhatti, (2011). Improving bio-ethanol yield: Using virginiamycin and sodium flouride at a Pakistani distillery. African Journal of Biotechnology, 2011. 10(53): p. 11071-11074.

[17] Willaert, R. and A.N. Viktor, Primay beer fermentation by immobilized yeast - a review on flavor formation and control strategies. Journal of Chemical Technology & Biotechnology, 2006. 81: p. 1353-1367.

[18] Walker, G.M. and P.V. Dijck, Physiological and molecular responses of yeasts to the environment, p. 111-152. *In* Querol, A. and G. Fleet (eds.), Yeasts in food and beverages, Springer-Verlag Berlin, Germany, 2006.