International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 1, p. 455-464, 2020

RESEARCH PAPER OPEN ACCESS

Enhanced yeast growth and bioethanol production using molasses and fermentation medium as substrates

Shafique Ahmed Rind[1], Fateh Muhammad Soomro[1], Agha Asad Noor[2], Atta Hussain Rind*[1]

[1]*Shah Abdul Latif University, Khairpur Mir's, Sindh, Pakistan*

[2]*University of Sindh, Jamshoro, Pakistan*

Key words: Yeast growth, molasses and fermentation medium, ethanol production.

http://dx.doi.org/10.12692/ijb/16.1.455-464 Article published on January 30, 2020

Abstract

The ethanol derived by the use of microorganisms during industrial fermentation process has been used as an alternate source of liquid energy and a substitute of high-cost petrol. *Saccharomyces cerevisiae* is best known microbial source due to its major impact on economic and social importance, rapid conversion of sugar, high growth and fermentation rate, high genetic stability and tolerance in stressful conditions. This work revealed that maximum growth of test strain on WL-nutrient broth, 0.8 ml inoculum size, 5.0 pH, and 32°C at 120 rpm after 72 hours. Glucose, sucrose; arginine, asparagine, glutamic acid; pantothenic acid, thiamine and riboflavin were the best organic sources for enhanced growth of test strains whereas calcium chloride, magnesium chloride, potassium chloride, ammonium chloride, ammonium sulphate, magnesium sulfate, manganese sulphate, potassium di-hydrogen phosphate, di-Potassium hydrogen phosphate, urea, peptone, yeast nitrogen base were the best chemicals for increased growth of test strain at their respective concentrations per 100ml. of WL-nutrient broth. The effect of alcohols showed that the test strain Cs tolerated higher concentration of ethanol as compared to the methanol and isopropanol. When studied the comparative growth, it was observed that the synthetic growth medium showed increased growth as compared to other media used. Ethanol production from clarified molasses and mixed with growth medium at different concentrations revealed 24.91% and 15.7% at sugar concentration 44 and 35.5% and sugar consumption 33.26 and 26.61% respectively.

*** Corresponding Author:** Atta Hussain Rind ✉ attahussain243@gmail.com

Introduction

Currently, the vehicles are more dependent on biofuels due to its storage capacity more significantly liquid fuels. The biofuel utilization depends upon the feedstock availability that depends upon the favorable climatic conditions, particular area of land and the crops for energetic yield. The advancement of this technology in national standards has been enhanced by investors and establishments. That's why the continuous efforts have been taken to uplift the high standard of biofuel. The greater portion of bioethanol is now marketed for better used in beverages, solvents and also some important used in different industrial processes (WWI, 2006). Recently, Brazil is the main supplier of ethanol that extends to half of total world's business of liquid renewable biofuels followed by Pakistan, USA, South Africa, Ukraine and others but the trade ratio is significantly lesser than Brazil. On contrary, Pakistan is also among the larger supplier of ethanol to the European countries due to the feedstock e.g. sugarcane crop. Many other regions grow sugarcane as cheaper feedstock like Africa, Latin America, and Asia plan to extend their yield for worldwide trade (WWI, 2006). Moreover, world's power requirement and the reduction of oil reserves gained access to progress the alternative energy resources from renewable resources (Saxen *et al.,* 2009) such as ethanol, a significant alternate of biofuel (Phouong, *et al.,* 2014), which is used to achieve the goals of energy crises either directly or mixed with gasoline (Ward, *et al.*, 2006; Manada *et al.,* 2015; Sanchez, *et al,*. 2008).

It is also used industrial solvent, cleansing agents, preservatives, an additive, fuel volume extender and as octane enhancer (Dung *et al.,* 2012; Woodson *et al.*, 2008). Sugarcane is one of the best known raw materials for bioethanol fermentation [9] among others that include corn cereals, wheat, municipal waste, Maize. Currently the production of 2nd generation biofuels from paper waste, 3rd generation from algal source whereas the 4th generation bioethanol are progressing from vegetable oil (Balat *et al.,* 2008; Byadgi *et al.,* 2016). *Saccharomyces cerevisiae* is a model microorganism being used for better fermentation of ethanol (Hashimoto *et al.,* 2005; Mobini-Dehkordi *et al.,* 2011). The essence of ethanol production valued because of high proficiency and low environmental effects, less emission of volatile compounds and the reduction of greenhouse gases emissions (Hahn-Hagerdal, *et al.*, 2006; Wyman, 1990; Periyasamy, 2009). Globally, the greater extent of the crude oil has been focused to use as a significant source of energy that may the environmentally acceptable. It has been observed that world is focusing the innovative technologies because of their benefit to the man kind in terms of minimizing effects of fuel energy, enhanced economic status, better and cleaned environment and inexpensive resources for the production of biofuels.

As the cost of petroleum products increases day by day the need of bioethanol emerges in the under developing countries. The work on bioethanol has been successfully done in the advanced countries for better quality, better use in vehicles in the form of E100, E80 and so on. This work is done to create the opportunity based production of biofuel in Pakistan to overcome the crises of petrol prices. This study still need the piot scale production, which is only accomplished with the generation of public sector and private sector agencies.

Keeping the significance of biofuel and its market importance, the international organizations in the 8th conference of Biofuels & Bioeconomy-2017 proclaimed that the biofuels are unaffected part of bio-industry (Biofuels & Bioeconomy *et al.*, 2018). The use of biofuel energy sources has environmental impacts such as air, water, and soil pollution at extraction sites, ducts, and refineries and the anthropogenic mobilization of millions of carbon tons that is the reason of global warming (Platon, *et al.,* 2017).

Aims And Objectives

Keeping the long term crises of high rates of petrol, this study was designed with the aim to produce bioethanol from the cheaper source of plant origin e.g. sugarcane molasses and the synthetic fermentation medium in different concentrations. It also explores the new trend of simpler technology to achieve the better yield of the bioethanol.

Materials and methods

Optimization studies for yeast growth

The commercial strain of *Saccharomyces cerevisiae* strain Cs was rehydrated in sterile distilled water and kept for incubation at 37°C for 2 hours and cultured in nutrient broth (Oxoid CM0001). Sub-culturing was also done on nutrient agar after 12, 24, 48 and 72 hours. Finally they were transferred to the WL-nutrient broth (WLNB) of Oxoid (CM050), absorption was recorded at 600 nm. Cell count was determined on both agar media (Sanders, 2012).

Later on yeast culture inoculated in WL-nutrient broth for physical and chemical optimization parameters including the effect of age, inoculum size, temperature with little modifications in the method of (Inan, *et al.,* 1999; Duhan *et al.,* 2013; AL-Sa'ady *et al.*, 2014), pH (Udhayaraja *et al.,* 2012) and agitation speed (Bernardo *et al.,* 2005, Rosma *et al.,* 2006), sugars, amino acids, vitamins and other minerals at absorption 600nm.

Preparation of fermentation medium for ethanol production

Clarified molasses (100 ml) in 300ml Erlenmeyer flask was used for ethanol production (I) whereas a mixture of molasses and growth medium was prepared in 20, 40, 80 in 80, 60, 20ml ratio respectively to make fermentation medium for ethanol production (II) and later Brix of cane molasses and fermentation medium was determined by Hana Brix meter HI96801 Digital Refractometer 0-85% (Shaheen, 2010).

Ethanol production (I and II)

Ethanol I was produced from sterile, cooled pretreated molasses whereas the ethanol II was produced by mixture of fermentation medium and molasses (80:20, 60:40 and 20:80ml).

Respectively with pH 5.0, 0.8ml inoculum size at 32°C, 120 rpm for 48h. Fermented mash was distilled at 78°C controlled temperature and the percentage was determined by alcohol density meter (DMA 35).

Results & discussions

Saccharomyces cerevisiae (strain Cs) was activated in nutrient broth and WL-nutrient broth. Total viable count and cultured on both agars that revealed 290,000 and 331,000 cell per ml during optimization of growth after every 12 hours in WL-nutrient broth revealed the increased growth after 72 hours post.

Incubation at A_{600} (Fig.1), inoculum size 0.8ml in 100ml of WLNB (Fig.2), pH 5.0, temperatures 32°C, agitation speed 120 rpm in orbital shaker incubator (Table 3-5), 1mg glucose and sucrose, 0.1mg of arginine, asparagine and glutamic acid and 0.1mg of thiamine, riboflavin and pantothenic acid (Table.6-8) and minerals (Sodium chloride, manganese chloride, di-potassium hydrogen phosphate and yeast extract) at 0.4mg whereas (Calcium chloride, potassium chloride, magnesium chloride, ammonium chloride.

Ammonium sulfate, magnesium sulphate, potassium phosphate, potassium di-hydrogen phosphate, urea, peptone, yeast nitrogen base at 0.2mg / 100ml respectively. The optimized concentrations of all chemicals, sugars, amino acids and vitamins were composed to prepare a synthetic growth medium (Table 1). Ethanol production was obtained pretreated sugar cane molasses (Ethanol-I, II) and from fermentation medium at various concentrations/ratio respectively (Table 2-3).

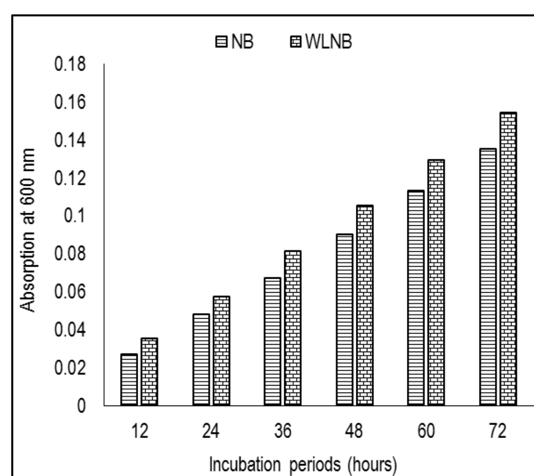

Fig. 1. Determination of maximum growth of test strain Cs on various incubation periods (age of inoculum) in modified WL- nutrient broth, 1 ml inoculum, at pH 5, 37°C, 100 rpm, A_{600}.

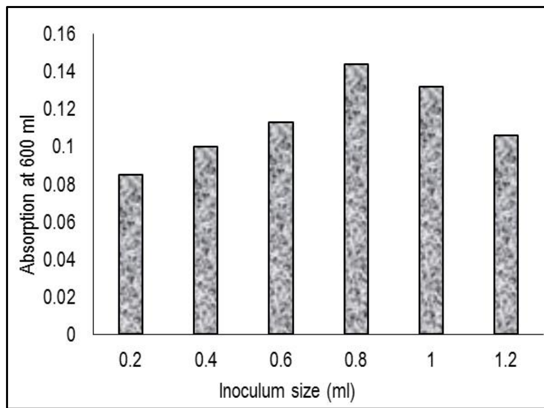

Fig. 2. Determination of maximum growth of test strain Cs on modified WL nutrient broth at various incubation periods (age of inoculum) in modified WL-nutrient broth, 1 ml inoculum, at pH 5, 37°C, 100 rpm, A_{600}.

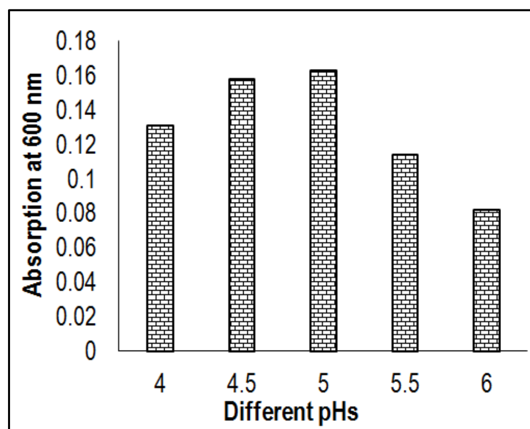

Fig. 3. Determination of maximum growth of test strain Cs on various pH, in modified WL nutrient broth at pH 5, 37°C, 0.8 ml inoculum size, 100 rpm after 72 h incubation at A_{600}.

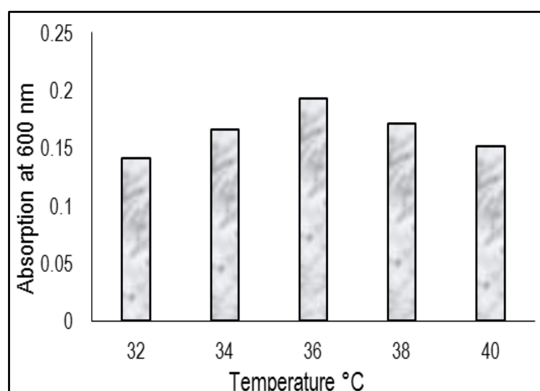

Fig. 4. Determination of maximum growth of test strain Cs on various temperatures in modified WL nutrient broth at pH 5, 37°C, 0.8ml inoculum size, 100 rpm after 72h incubation at A_{600}.

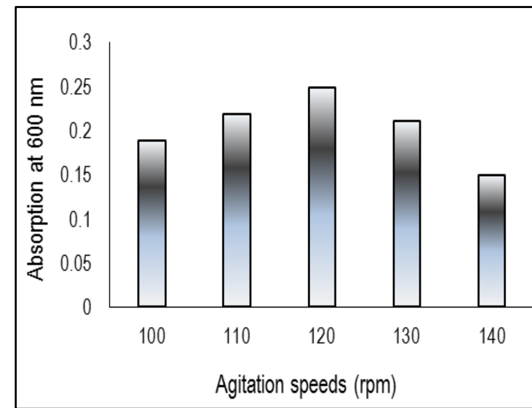

Fig. 5. Determination of maximum growth of test strain Cs on various agitation speeds in modified WL nutrient broth at pH 5, 36°C, 0.8ml inoculum size, 100 rpm after 72h incubation at A_{600}.

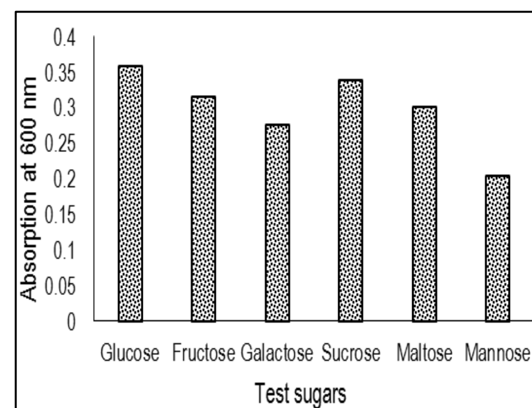

Fig. 6. Determination of maximum growth of test strain Cs on various sugars (1mg / 100ml) in modified WL nutrient broth at pH 5, 36°C, 0.8ml inoculum size, 120 rpm after 72h incubation at A_{600}.

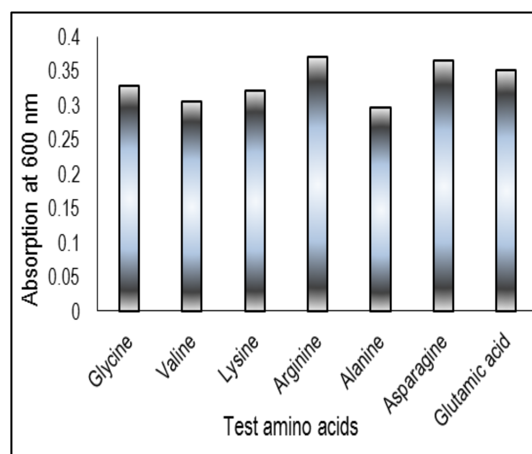

Fig. 7. Determination of maximum growth of test strain Cs on various amino acids (0.1mg/100 ml) in modified WL nutrient broth at pH 5, 36°C, 0.8ml inoculum size, 120 rpm after 72h incubation at A_{600}.

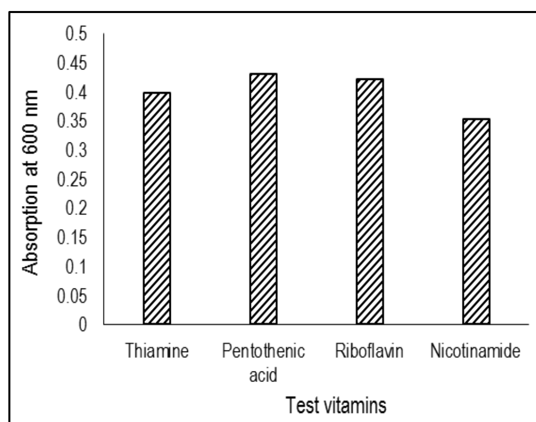

Fig. 8. Determination of maximum growth of test strain Cs on various vitamins (0.1mg/100 ml) in modified WL nutrient broth at pH 5, 36°C, 0.8ml inoculum size, 120 rpm after 72h incubation at A_{600}.

Table 1. Chemical composition of fermentation medium (GM).

Chemicals	Composition g / L
Yeast extract	4
Magnesium chloride	4
Di-potassium hydrogen phosphate	4
Calcium chloride	2
Potassium chloride	2
Magnesium chloride	2
Ammonium chloride	2
Ammonium sulphate	2
Manganese sulphate	2
Potassium phosphate	2
Potassium di-hydrogen phosphate	1
Urea	2
Peptone	5
Yeast nitrogen base	2
Glucose	10
Thiamine	0.1
Riboflavin	0.1
Pantothenic acid	0.1
Arginine	1
Asparagine	1
Glutamic acid	1
Agar-agar	12
pH	5.0

Table 2. Ethanol production (I) from sugar cane molasses by test strain Cs. at pH 5, 32°C, inoculum size 0.8ml, 120 rpm after 48 h incubation at A_{600}

Substrate	Sugar % (a)	Ethanol-production (%)	Sugar present after fermentation (b)	a-b = total sugar consumed (%)
Molasses	44	24.91	10.74	33.26

Table 3. Ethanol production (II) from sugar cane molasses by test strain Cs at pH 5, 32°C, inoculum size 0.8 ml, 120 rpm after 48 h incubation at A_{600}.

Substrate (Molasses and GM ratio - ml)	Sugar % (a)	Ethanol production (%)	Sugar present after fermentation (b)	a-b= total sugar consumed (%)
20-80	9.3	5.81	2.24	7.06
40-60	17.1	10.3	4.17	12.93
80-20	35.4	15.7	8.79	26.61

Ethanol from cane molasses is a good source of liquid energy for industries and vehicles due to its low cost and the use animal feed (Ghorbani *et al.,* 2011; Paciello *et al.,* 2014; Arshad *et al.,* 2008; Kopsahelis *et al.,* 2009; Xandé *et al.,* 2010). Yeasts are unique microbial sources such as *Saccharomyces cerevisiae* (Nielsen *et al.,* 2013). Nutrients supply to this yeast at various time periods strengthen the yeast growth, increase the number of cells and enhances the rapid metabolic activity (Samsuri, *et al.,* 2010). WL-nutrient agar supports the uptake of nutritional requirements through mitochondrial enzymes and hereditary traits by the genes. Growth medium revealed the higher growth due to the active proton symport and facilitated diffusion mechanisms. Amino acids are assimilated and transported and play a structural role. Vitamins help in decarboxylation, acetylation, redox reactions and transfer of methyl groups (Noor *et al* 2008, Noor *et al.,* 2012). Optimization studies were used to enhance yeast growth and ethanol production (Janani *et al.,* 2013; Swain *et al.,* 2013). In our study optimization parameters confirm the maximum growth due to the activation of transcriptional control elements. The age of inoculum revealed that growth increases after 48 hours and reached at maximum level up to 72 hours due to their rapid enzymatic metabolism that reaches its log phase. The observation revealed that 0.8% inoculum grows well due to the active uptake of nutrients and appropriate oxygen supply in the medium. The size of inoculum in ethanol fermentation is important for greater fermentation yield (Alegre, *et al.,* 2003).

Temperature supports the inoculum development at 30-35°C (Noor *et al.,* 2003). Increase in temperature denature microbial enzymes and cause inability of yeast's regulatory mechanism for the tolerance and

decrease in ethanol production (Shah *et al.,* 2010; Lin *et al.*, 2012). The findings revealed the maximum growth at 32°C which may be due to the combination of enrichment factors of the in WLNB, oxygen content and the agitation speed. This observation of sugar consumption and ethanol production is well accorded with (Banat *et al.,* 1995; Rajoka *et al.,* 2005). Our studies revealed the variation in growth that reached to maximum at pH 5.00. This may be due to the enzyme production, metabolic activity and un-dissociated form of weak acid. Agitation provides a mechanical set up for compressed air that provides uniform distribution of nutrients and oxygen supply.

Aerated medium supports the greater biomass because agitation provides gaseous phase of oxygen into the liquid phase to the growing cells needed for mitochondrial cyclic metabolism the equal concentrations of the nutritional ingredients to all cellular components (Noor *et al.,* 2005; Inparuban, *et al*, .2009). Yeast can utilize glucose, fructose, maltose or sucrose as their main carbon source. The increase of sugar beyond 15% can inhibit the cellular activity (Stenberg, *et al*., 2000). Glucose supports the yeast growth in non-fermentable carbon source, which induce various signal transduction pathways and enhances ATP for cell mass biosynthesis (Walker, 2000). Our result is in agreement of (Noor *et al.,* 1996). Yeast utilizes nitrogen for growth and activity. The growth in nitrogenous sources supports greater ethanol yield in ammonium sulphate, potassium nitrate. The findings indicated the maximum growth in yeast nitrogen base with ammonium sulphate, urea and peptone.

Optimization of chemicals help determine osmotic stress (Logothetis, *et al.,* 2007; Mukherjee *et al.,* 2010) that play an important role in the cellular metabolism. Chloride ions help in fluid and electrolyte balance, manganese helps in intracellular regulation, calcium helps in cell cycle regulation and implicated in transitional form lag to exponential phase. Potassium and magnesium ions establish metallic cationic environment and thus potassium (K) calcium (Ca), magnesium (Mg) support growth and enzyme system as co factor. Sulphur helps in biosynthesis of sulphur containing amino acids, prevention of browning reaction and phosphates play a vital role in carbohydrate metabolism and yeast growth. Molasses possesses lots of impurities that could be toxic because different chemicals such as fungicides, insecticides and herbicides heavy metals and also fertilizers (Reed, *et al.,* 1988, Pérez-Torrado, 2004). A new modified substrate was formulated with varying concentrations of sugar cane molasses and growth medium to constitute a fermentation medium in accordance to that provides enrichment for maximum growth and protection of yeast growth from toxic effects of molasses.

The rate of ethanol production depends upon the sugar concentration. High and low sugar on ethanol fermentation and the productivity resulting the increased osmotic pressure due to the starvation of yeast cells. Our observations revealed the ethanol production after 48 h due to the cell-specificity cell age and the optimized ratio carbon, nitrogen source and other nutrients (Paciello *et al.,* 2009; Paciello *et al.*, 2019). Our results of ethanol production at 32°C after 48h are in agreement of (Ballesteros, *et al.,* 2004; Aldiguer *et al.,* 2004, Phisalaphong *et al.,* 2005).

Conclusion

From the observations it is concluded that activated culture of *Saccharomyces cerevisiae* show well growth after 72h starting after 46 h incubation. The WL-nutrient medium was the best medium for higher growth then the simple nutrient agar. While optimization studies the maximum growth appeared after 72h, 0.8ml inoculum size at 32°C, 120 rpm in WL-nutrient broth with pH 5.0. Glucose and sucrose, arginine, asparagine and glutamic acid, Biotin, thiamine and pantothenic acid were observed as best carbon, nitrogen sources and growth factor respectively. Clarified substrate and fermentation medium with suitable optimization parameters will increase the yeast growth and the fermentation ability of the teats strain. It is also concluded that clarified molasses and the fermentation medium (molasses and growth medium) at 80-20 ratio was known as best substrate respectively.

References

Aldiguer AS, Alfenore S, Cameleyer X, Goma G. 2004 .Synergistic temperature and ethanol effect on *Saccharomyces cerevisiae* dynamic behavior in ethanol bio-fuel production. Bioprocess Bio system Engineering volume **26,** No. 4, 217-222.

Alegre RM, Rigo M, Jokes I. 2003. Ethanol fermentation of a diluted molasses medium by *Saccharomyces cerevisiae* immobilized on chrysotile. Brazalian Archieve of Biology and Technology volume **46,** No. 4, 751-757.

AL-Sa'ady AJR. 2014.Optimization of invertase production from *Saccharomyces cerevisiae* by solid state fermentation. Current Research in Microbiology and Biotechnology volume **2,** No. 3, 373-377.

Arifa T, Aftab M, Farasat T. bio-07. J. Applied 2010. Effect of cultural conditions on ethanol production by locally isolated Saccharomyces cerevisiae Pharmacology volume **3,** No. 2, 72-78.

Arshad M, Khan ZM, Khalil-ur-Rehman, Shah FA, Rajoka MI. 2008. Optimization of process variables for minimization of byproduct formation during fermentation of blackstrap molasses to ethanol at industrial scale. Letters in Applied Microbiology volume **47,** No. 5, pp. 410-414..

Balat M, Balat H, Oz C. 2008. Progress in bioethanol processing. Progress in Energy and Combustion Science volume 34, No. 5, 551-573.

Ballesteros M, Oliva JM, Negro MJ, Manzanares P, Ballesteros I. 2004. Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with *Kluyveromyces marxianus* CECT 10875. Process Biochemistry volume **39,** No. 12, 1843-1848.

Banat IM, Marchant R. 1995. Characterization and potential industrial applications of five novel, thermo-tolerant, fermentative yeast strains. World Journal of Microbiology and Biotechnology volume **11,** No. 3, 304-306. https://doi.org/10.1007/BF00367104.

Bernardo O, Silva-Santisteban Y, Francisco. 2005. Agitation, aeration and shear stress as key factors in inulinase production by *Kluyveromyces marxianus.* Enzyme and Microbial Technology, volume **36,** No. 5-6, 717-724.

Biofuels & Bioeconomy. 2018. The 8th International Conference on Biofuels, Bioenergy & Bioeconomy. Available from http://biofuels-bioeconomy.conferenceseries.com.

Byadgi SA, Kalburgi PB. 2016. Production of Bioethanol from Waste Newspaper. International Conference on Solid Waste Management, Procedia Environmental Sciences, volume **35,** 555-562. https://doi.org/10.1016/j.proenv.07.040.

Duhan JS, Kumar A, Tanwar SK. 2013. Bioethanol production from starchy part of tuberous plant (potato) using *Saccharomyces cerevisiae* MTCC-170. African Journal of Microbiology Research volume **7,** No. 46, 5253-5260.

Dung NTP, Tuong NH, Quang PH. 2012 .Testing of ethanol fermentation in sugarcane juice by thermotolerant yeasts. The 5th Satellite Seminar of JSPS-NRCT, Asian Core Program, 18-20.

Ghorbani F, Younesi H, Sri AE, Najafpour G. 2011 .Cane molasses fermentation for continuous ethanol production in an immobilized cells reactor by *Saccharomyces cerevisiae.* Renewable Energy volume **36,** No. 2, 503-509.

Hahn-Hagerdal B, Gable M, Gorwa-Graslund MF, Liden G, Zacchi G. 2006. Bioethanol: The fuel of tomorrow from the residues of today. Trends in Biotechnology volume **24,** No. 12, 549-556.

Hashimoto S, Ogura M, Aritomi K, Hoshida H, Nishizawa Y, Akada R. 2005. Isolation of auxotrophic mutants of diploid industrial yeast strains after UV mutagenesis. Applied and Environmental Microbiology volume **71,** No. 1, 312-319, http:// doi.org/10.1128/AEM.71.1.312-319.

Inan M, Chiruvolu V, Eskridge KM, Vlasuk GP, Dickerson K, Brow S. 1999 .Optimization of temperature, glycerol and pH conditions for fed-batch fermentation process for recombinant hookworm (*Ancylostoma caninum*) anticoagulant peptide (AcAP-5) production by *Pichia pastoris*. Enzyme and Microbiology Technology volume **24,** No. 7, 438-445.

Inparuban K, Vasantharuba S, Balakumar S, Arasaratnam V. 2009. Optimization of culture conditions for Baker's yeast cell mass production- a preliminary study. Journal of Science Eastern University Sri Lanka volume **6,** No. 1, 34-45.

Janani K, Ketzi M, Megavathi S, Vinothkumar D, Babu RNG. 2013. Comparative Studies of Ethanol Production from different fruit wastes using *Saccharomyces cerevisiae*. International Journal of Innovative Research in Science, Journal Engineering and Technology volume **2,** No. 12, pp. 7161-7167,

Kopsahelis N, Nisiotou A, Kourkoutas Y, Panas P, Nychas GJ, Kanellaki M. 2009. Molecular characterization and molasses fermentation performance of a wild yeast strain operating in an extremely wide temperature range. Bio resource Technology volume **100,** No. 20, 4854-4862.

Landi C, Paciello L, de Alteriis E, Brambilla L, Parascandola P. 2001. Effect of auxotrophies on yeast performance in aerated fed-batch reactor. Biochemical Biophysical Research Communication volume **414,** No. 3, 604-611.

Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H. 2012 .Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. Biomass and Bioenergy volume **47,** 395-401. http://doi.org/10.1016/j.biombioe.2012.09.019.

Logothetis S, Walker G, Nerantzis ET. 2007. Effect of salt hyperosmotic stress on yeast cell viability. Proc Nat Sci Matica Srpska Novi Sad volume **113,** 271-284.

Macedo IC, Seabra JEA, Silva JEAR. 2008. Greenhouse gases emissions in the production and use of ethanol from sugarcane in Brazil: the 2005/2006 averages and a prediction for 2020. Biomass and Bioenergy volume **32,** No. 7, 582-595.

Manada P, Kathale N. 2015. Production of ethanol from mahua flower (*Madhuca latifolia* L.) using *Saccharomyces cerevisiae*- 3044 and study of parameters while fermentation. Journal of Research in Science and Technology, volume **1,** No. 9, 6-10.

Mobini-Dehkordi M, Nahvi I, Zarkesh-Esfahani H, Ghaedi K, Tavassoli M, Akada R. 2011. Characterization of an interesting novel mutant strain of commercial *Saccharomyces cerevisiae*. Iranian Journal of Biotechnology, volume **9,** No. 2, 109-114.

Mukherjee K, Banik AK. 2010. Effect of trace elements on biosorption of Hg2+ by Hg2+ tolerant. *Saccharomyces cerevisiae* A100. International. Journal of Pharmaceutical and Biosciences, volume **1,** No. 2, 1-10.

Nielsen J, Larsson C, Maris VA, Pronk J. 2013. Metabolic engineering of yeast for production of fuels and chemicals. Current Opinion in Biotechnology, volume **24,** No. 3, 398-404.

Noor AA, Dahot MU. 2008. Optimization studies of yeast feed for enhanced growth for ethanol production by shake flask method. Sindh University Research Journal (Sci. Ser.), Volume **40,** No. 2, pp. 41-50.

Noor AA, Hameed A, Bhatti KP, Tunio SA. 2003 Bio-ethanol fermentation by the bioconversion of sugar from dates by *Saccharomyces cerevisiae* strains ASN-3 and HA-4. Biotechnology, volume **2,** No. 1, 8-17.

Noor AA, Hameed A, Bhutto MA. 2005. Enhanced growth biotechnology of yeast for alcoholic fermentation. Biotechnology, volume **4,** No. 1, 69-75.

Noor AA, Hameed A, Dahot MU. 2012. Yeast Feed (II) For Enhanced Growth for Ethanol Production. Sindh University Research Journal (Science Series), volume **44,** No. 1, 97-104.

Noor AA, Hameed A, Ghumro PB. 1996 .The 96 hours multistep screening of sugar fermenting yeast *Saccharomyces cerevisiae* strain KA-1. J. Pure and Applied Science, volume **18,** No. 1, 23-33.

Paciello AP, Parascandola P, Landi C. 2014. Auxotrophic *Saccharomyces cerevisiae* CEN.PK strains as new performers in ethanol production. Chemical Engineering Transactions, volume **38,** 463-468.

Paciello L, Andrès I, Zueco J, Bianchi MM, de Alteriis E, Parascandola P. 2010. Expression of human interleukin-1b in *Saccharomyces cerevisiae* using PIR4 as fusion partner and production in aerated fedbatch reactor. Ann. Microbiol., Volume **60,** 719-728.

Paciello L, Romano V, de Alteriis E, Zueco J, Parascandola P. 2009. Performance of the auxotrophic *Saccharomyces cerevisiae* BY4741 as host for human Interleukin-1β production in an aerated fed-batch reactor. Microbial Cell Factories, volume **8,** 1-13.

Peña-Castro JM, del Moral S, Núñez-López L, Blanca E, Barrera-Figueroa, Amaya-Delgado L. 2017, Biotechnological Strategies to Improve Plant Biomass Quality for Bioethanol Production**.** BioMed Research International, volume **2017,** 1-10.

Pérez-Torrado R. 2004. Estudio y mejora Del proceso de producción industrial de levaduras vínicas. PhD thesis, Universitat de València. Valencisa, Spain.

Periyasamy S. 2009. Production of Bio-ethanol from Sugar Molasses Using Saccharomyces cerevisiae. Modern and Applied Sciences, volume **3,** No. 8, 32-37.

Phisalaphong M, Srirattana N, Tanthapanichakoon W. 2006. Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation. Biochemical Engineering Journal, volume **28,** No. 1, 36-43.

Phouong DNT, Huu TN, Xuan PH. 2014. Study on Ethanol fermentation conditions from molasses by thermo-tolerant yeasts. International Journal of Business and Applied Science, volume **1,** No. 1, 13-22.

Reed G, Nagodawithana TW. 1988. Technology of Yeast Usage in Winemaking. American Journal of Enology and Viticulture, volume **39,** No. 1, 83-90.

Rosma A, Ooi KI. 2006. Production of Candida utilizes biomass and intracellular protein content: Effect of agitation speed and aeration rate. Malaysian J. Microbiol. Volume **2,** No. 2, pp. 15-18.

Samsuri M, Gozan M, Hermansyah H, Prasetya B, Nasikin M, Watanabe T. 2010. Ethanol production from bagasse with combination of cellulose cellubiase. In: simultaneous saccharification and fermentation (ssf) using white rot fungi pre-treatment. Journal of Chemical and Natural Resources Engineering, volume **3,** 20-32.

Sanchez OJ, Carlos AC. 2008. Trends in biotechnological production of fuel ethanol from different feedstock. Biores. Technol., Volume **99,** No. 13, pp. 5270-5295.

Saxena RC, Adhikari DK, Goyal HB. 2009. Biomass-based energy fuel through biochemical routes. A review. Renewable and Sustainable Energy Reviews, volume **13,** 167-178.

Shah SFA. 2010. Enhanced production of ethanol from sugar cane molasses through thermotolerant *Saccharomomy cescerevisiae* cell. Ph. D. Thesis, Mehran University of Engineering and Technology, Jamshoro, Pakistan, 1-132.

Shaheen A. 2010. Production of ethanol from molasses using thermotolerant *Kluyveromyces marxianus*. Ph.D. thesis, Mehran University of Engineering and Technology, Jamshoro, Pakistan 1-187.

Stenberg K, Bollók M, Réczey K, Galbe M, Zacchi, G**.** 2000. Effect of substrate and cellulose concentration on simultaneous saccharification and fermentation steam-preheated soft wood for ethanol production. Biotechnology Bioengineering, volume **68,** No. 2, 204-210.

Swain MR, Mishra J, Thatoi H. 2013. Bioethanol Production from Sweet Potato (*Ipomoea batatas* L.) Flour using Co-Culture of *Trichoderma* sp. and *Saccharomyces cerevisiae* in Solid-State Fermentation, Brazilian archives of Biology and Technology, volume **56,** No. 2, 171-179.

Udhayaraja P, Narayanan JS. 2012. Optimization for production of bioethanol using sorghum stovar by *Saccharomyces cerevisiae*. International Journal of Research in Pure and Applied Microbiology, volume **2,** No. 4, 64-67.

Walker GM. 2000 .Encyclopedia of Microbiology, Academic Press, volume **4,** p. 939-953.

Ward OP, Singh A. 2006**.** Microbial technology for bioethanol production from agricultural waste. In: Ray RC Ed. Microbial Biotechnology in Agriculture and Aqua culture, vol. 1, Science Publishers, Enfield, New Hampshire, p. 517-557,.

Woodson M, Jablonowski CJ. 2008. An economic assessment of traditional and cellulosic ethanol technologies. Energy Sources, Part B., volume **3,** 372-383.
http://doi:10.1080/15567240701232527.

WWI. 2006. Biofuels for transportation, global potential and implications for sustainable agriculture and energy in the 21st century, Management of Environmental Quality, volume **19,** No. 1, p. 3-451. https://doi.org/10.1108/meq.2008.08319aae.005.

Wyman CE, Hinman ND. 1990. Ethanol: Fundamentals of ethanol production from renewable feed stocks and use as a transportation fuel. Applied Biochemistry and Biotechnology, Volume **24,** No. 25, 735-753.

Xandé X, Archimède H, Gourdine JL, Anais C, Renaudeau D. 2010. Effects of the level of sugarcane molasses on growth and carcass performance of Caribbean growing pigs reared under a ground sugarcane stalks feeding system. Tropical Animal Health and Production volume **42,** No. 1, 13-20.