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Increasing Ethanol Production on Commercial Scale Using Sugarcane Molasses at very High Gravity Technique

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ABSTRACT

Sugarcane is one of the main crops in Pakistan. Molasses is the byproduct of sugar industry that is further processed for ethanol production. Therefore, the main aim of this study is to produce enhanced quality and quantity of ethanol using by product (molasses) collected from different sugar mills. Experiments were performed to optimize the quantity and quality of bioethanol produced during this study. The experiments were conducted by varying aeration levels and molasses brix during fermentation. The pH was maintained at 3.8-4.8 in pre-fermenters by the addition of Sulfuric Acid. Additionally, Urea was also introduced in pre-fermenters for the rapid cell count growth and viability. The temperature of the pre-fermenter was maintained from 29ºC - 31ºC, while the temperature of fermenter was controlled in the range of 29ºC - 33ºC. The viable cell counts at 0.45vvm was observed highest 415million/mL The viable cell count was noticed 18, 21 and 23% at 0.25, 0.35 and 0.45 vvm, respectively. The ethanol production was recorded at 8.8% at 34 ◦Brix without aeration, while using aeration environment the ethanol production reached to 10.7%. The ethanol production at aeration rates 0.25, 0.35, and 0.45 vvm was increased 19, 17 and 15%, respectively as compared with non-aeration environment. The optimum production of ethanol was achieved at 29ºCand 5.5 pH.

Keywords: Sugarcane; molasses; fermentation; ethanol; efficiency.

1. INTRODUCTION

In renewable energy projects, the investment level has increased tremendously throughout the globe in the last ten years. The investment in the year 2007 was 104 billion dollars, whereas in 2017 it reached 279.8 billion dollars(Kamran, Fazal, & Mudassar, 2020).

In renewable energy projects, the investment level has increased tremendously throughout the globe in the last ten years. The investment in the year 2007 was 104 billion dollars, whereas in 2017 it reached 279.8 billion dollars(Kamran, Fazal, & Mudassar, 2020). The increase in investment in renewable energy doubled the installed generation capacity in the world. The installed renewable energy power potential was 1070 gigawatt in 2007, while the installed renewable energy power generation capacity increased to 2195 gigawatt in 2017. Adherence to renewable energy options will reduce environmental degradation. Furthermore, different countries have set challenging targets for forthcoming years to increase the contribution of alternative energy in power generation capacity. Weather conditions and uncertainty in renewable energy resources make it difficult to design a scheme or algorithm to efficiently integrate with power generation systems. Therefore, various studies are done to address variations in renewable energy supply in recent years (Amamra, Meghriche, Cherifi, & Francois, 2017; Denholm, Brinkman, & Mai, 2018; Saez-de-Ibarra, Martinez-Laserna, Stroe, Swierczynski, & Rodriguez, 2016). Furthermore, the demand for energy is growing throughout the globe as the prosperity and wellbeing of people are closely related to the uninterrupted supply of energy at an affordable cost without causing any damage to flora and fauna. The energy consumption in the world is expected to rise by 28% in the period 2015 to 2040. Moreover, in Asia, energy consumption will rise to 51% in the same period. This 51% rise in energy consumption in Asia is the largest than any other region of the world. Presently more than 1.4 billion people lack access sot electricity, most people lack access to electricity belong to the African countries. In India alone more than 400 million people have no access to electricity while in Pakistan 25 million people are facing energy shortages. In Pakistan nearly 6500 MW electricity deficit was observed in 2017(Wakeel, Chen, & Jahangir, 2016).Currently, load shedding up to 18 hours a day has become a common practice in Pakistan that has adversely affected the economic growth and living standard of common people within the country. In rural areas of the country long breakdowns of electricity supply are frequently observed and people of rural areas are facing worst conditions. As renewable energy resources such as biomass, hydro solar, tidal, and geothermal and wind are abundant in Pakistan that maybe exploited. The renewable energy sources are sufficient to meet energy requirements(Ali, Jiang, & Khan, 2017).The utilization of fossil fuels for energy generation is increasing day by day. Therefore, fossil fuel resources are depleting at a rapid pace. Due to our over adherence with nonrenewable energy sources various environmental and health issues arise. Pakistan is an agricultural country and bio-waste produced in huge quantities that are improperly managed and discarded in the open areas. The improper handling of bio-waste cause serious environmental and health issues(Madhania, Muharam, Winardi, & Purwanto, 2019). The burning of crop residues in the field increases air contaminants in the form of SO_x and NO_x that further aggravate the environment within the vicinity. Besides that, municipal solid waste is thrown out in streets and nearby open areas where mosquitoes, flies, rodents and other various types of insects, animals, and birds gather. Therefore, there is a need to encourage efforts to fulfill the requirement of energy using renewable. As already mentioned, Pakistan is an agricultural country, and sugarcane is grown in large quantities. After sugar production, the black strap syrup is transported to distilleries to produce ethanol (C_2H_5OH) from it. The application of bioethanol as an alternative fuel will reduce over-dependence on fossil fuels and will result in lower CO_2 emissions. Fermentation sucrose-containing substances such as sugarcane(de Carvalho, Antunes, & Freire, 2016), sugar beet, fruits, starch containing materials including corn, cassava(Chao, Liu, Zhang, Zhang, & Tan, 2017)sweet potatoes(e Silva, Almeida, da Conceição Alvim-Ferraz, & Dias, 2018), lignocellulosic substances grass(Liu et al., 2017), paper waste(Byadgi & Kalburgi, 2016; Wang, Sharifzadeh, Templer, & Murphy, 2013), corn Cob(Boonchuay et al., 2018), wheat straw(Tomás-Pejó, Oliva, González, Ballesteros, & Ballesteros, 2009), and algae(Bibi et al., 2017)are performed for the production of bioethanol. Numerous efforts are done for the optimization of ethanol production, for the optimization of fermentation and distillation processes(Tgarguifa, Abderafi, & Bounahmidi, 2017).In some process optimizations, immobilized yeast has been used to increase the ethanol yield(Mohd Azhar et al., 2017). Whereas stirrers were also applied for enhanced production recovery of bioethanol. Through the application of stirrer, yeast is evenly distributed in the substrate and increases the contact time between substrate and yeast and thus enhances the fermentation efficiency.

Different researchers have performed computation fluid dynamic studies to understand the effect of mixing patterns on ethanol production on an industrial scale. The computational study of mixing within the fermenter and its effects on yield is an economical and efficient method of analysis the bioethanol production. The computational studies done on mixing are impeller layout (Rahimi, 2005), speed of rotational impeller flow rate and position of feed working fluid rheology and with different types of impellers(Sossa-Echeverria & Taghipour, 2015), the layout of propeller and rheology of working fluid(Wang et al., 2013).

1. MATERIALS AND METHODS

2.1 Microorganism and Culture Media

The microorganism (dry yeast) *Saccharomyces cerevisae* parental strain (Instant France) were purchased from local market. The developed inoculum *Saccharomyces cerevisiae* was used for fermentation of cane molasses throughout the experimental study.

2.2 Pretreatment of Molasses

Pretreatment of diluted molasses was done using conical bottom type settling tanks (Fig. 1). The conical bottom type settling tanks were specially designed for the removal of particulate matter and sludge. For the removal of sludge, ash and particulate matter sodium hexametaphosphate was used whereas the pH of diluted molasses was maintained 4.2 to 4.4 using commercial grade sulfuric acid that converted Ca^{2+} into calcium sulfate.

2.3 Preparation of Inoculum

The inoculum was prepared in 2m^3 capacity of a vessel when cell count reached 3.0x10^6 then inoculum was transferred to the pre-fermenter having 50m^3 capacity. Three pre-fermenters having 50m^{3}capacities of each pre-fermenter were used for the further propagation of yeast cells to maintain appropriate cell count and viability. Furthermore, this prepared inoculum was used for the fermentation of diluted molasses.

2.4 Fermentation of diluted molasses

The fermenter capacity of 250m^3 was used for the batch-wise experiments. The inside temperature of fermenter was maintained 30-33ºC. Due to hot climate plate type heat exchangers were used for the circulation of water to maintain the temperature of the fermenter in range of 30-33ºC. The mesh was re-circulated by making indirect contact with the re-circulated water. The electric pump used for the circulation of water was operated at 300rpm. The silica-based antifoaming agent was used for the controlling of foaming within the fermenter. Throughout the fermentation process the aeration rate was managed 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 (vvm) using air blower. The inoculum prepared in pre-fermenter was transported within the fermenter up to 24% of its capacity. The feeding time after the transportation of inoculum was maintained 18-19 hours. The feeding of diluted molasses in the fermenter was kept 33 Brix. The cell count and viability of cells were monitored every three hours by taking a sample from sample cock of the fermenter. The rise in fermenter level during feeding of diluted molasses was maintained 4.5 percent per hour. Eight fermenters were taken in operation for each run for the estimation of reproducibility.

2.5 Viability and Cell Count Analysis

The prepared inoculum in pre-fermentation was analyzed for the cell count and viability (Fig. 2). For the calculation of cell count hematocytometer was applied. The dilution of the collected samples was done using sterile saline solution 0.89%w/v NaCl whereas the viability of cell was observed through the application of methylene blue technique.

2.6 Distillation

The fermented mash from the vat was taken to distillation plant France (Fig. 1), having capacity of ninety thousand (90000) liters per day. Fermented mash was directed to the boiling column where steam from boiler was supplied to the boiling column to maintain the temperature of boiling column from 78-80ºC. The boiling column was operated under vacuum and the vacuum was applied through the condensers. Thus, the vapors collected from the top of the boiling column were collected from the top of the boiling column were condensed and cooled in the condensers. Then 25 percent of cooled ethanol was refluxed to boiling column while rest of the ethanol was taken to rectification column for the further increasing quality of ethanol product. After the rectification column the ethanol was directed to refining column for further treatment.

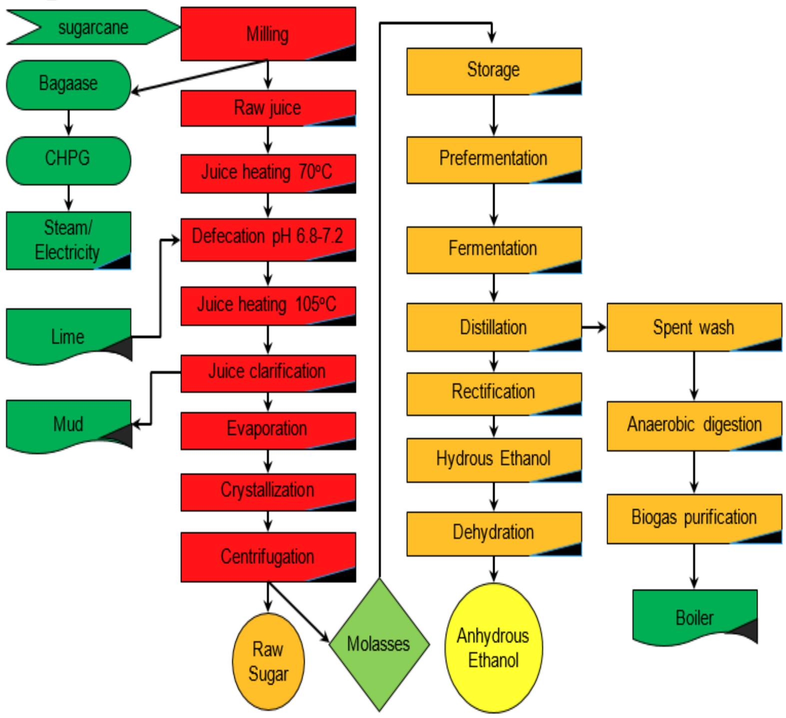

Fig.1 Flow chart of sugar, ethanol, and biogas from sugarcane.

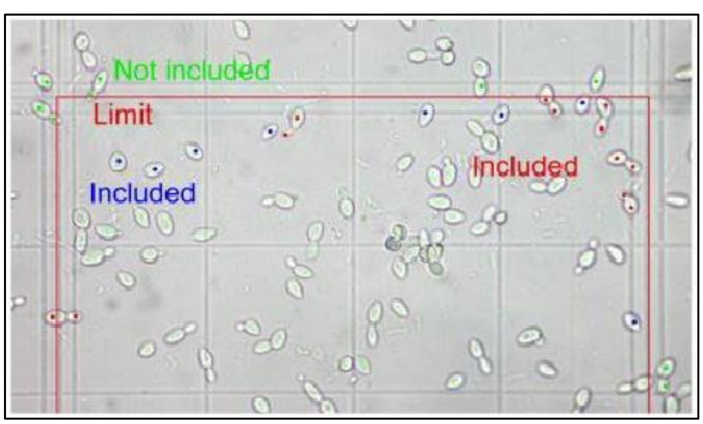

Fig. 2 Cell count and viability of cell.

2. RESULTS AND DISCUSSIONS

Different concentrations of dissolved solids having a range from 28 to 36 °Brix were selected for fermenter at high gravity molasses media. The prepared mash was taken into 250m^3 fermentation for 55 hours operation. The fermentation of prepared mash was done with the supply of air and without the supply of air during the fermentation process on an industrial scale to enhance ethanol production. The conventional treatment of molasses produced less ethanol as compared to very high gravity fermentation. High gravity fermentation produced ethanol in range of 10 to 11% v/v percent alcohol throughout the experimental analysis of production, however conventional method for ethanol production produced 6 to 9 % v/v alcohol. The difference in alcohol production between high gravity and conventional methods of ethanol production was nearly 3 to 4 percent. However, the production of ethanol was 13.31% from 27% TRS at rate of aeration 0.2 vvm. The high sugar level in molasses produced higher concentration ethanol during fermentation process. Furthermore, the results collected in this study are in good agreement with the results gathered by the researcher(Arshad, Hussain, Iqbal, & Abbas, 2017).

Fig. 3 shows the production of ethanol and residual sugar at different aeration rates and at 32 °Brix. The ethanol production and residual sugar without aeration were observed 9.4% and 34.6g/L content. The viable cell counts at 32 °Brix is shown in Fig.4the viable cell count at 0.45vvm was observed highest 415million/mL The viable cell count was noticed 18%, 21% and 23% at 0.25, 0.35 and 0.45 vvm respectively. Ethanol production was recorded 8.8% at 34 °Brix without aeration, while using aeration environment the ethanol production reached 10.7% v/v. The ethanol production at aeration rates 0.25, 0.35, and 0.45 vvm was increased 19%, 17% and 15% respectively as compared with non-aeration environment. The residual sugar was increased to 30 hours of fermentation and then started to decrease by up to 60 hours of operation (Fig. 5). With an increase in sugar level the viable cell count was increased up to 497 million/Ml. the °Brix level significantly affected the ethanol production, the increase in ethanol production and decrease in residual sugar is good indication of fermentation. The optimum ethanol yield was achieved 10.9 v/v at 38 ◦Brix with 0.25vvm aeration rate. At the aeration rate of 0.25, 0.35 and 0.45vvm was 17.6, 23.7 and 20.9 g/L respectively. Moreover, in non-aerated environment the residual sugar was observed 58.4g/L. Under non aeration conditions the viability of yeast cells declined 25% at 34 ◦Brix. The residual sugar was very high as compared with aerated conditions. Moreover, ethanol production was also improved by 18% by maintain 0.2vvm aeration conditions in fermenter. While production pf ethanol decreased at higher aeration rates. The excessive supply of aeration resulted decrease in ethanol production and acted as inhibitor by reducing S. cerevisiae, while as compared with high aerobic conditions more ethanol was produced at lower amount of dissolved oxygen in the fermenter. The residual sugar in non-aerated fermenter was recorded 40.8% and residual sugar in aerated fermenter was recorded 15.6% much lower than non-aeration environment. The maximum residual sugar reached 130.5 g/L during feeding (Fig.6). The yeast cells viability after 50 hours of operations was 81%. The acidity was low, and alcohol production was high in aerated environment as compared with non-aeration. Whereas the formation of alcohol and byproducts was higher under aeration environment and acidity was low at 0.21vvm as compared to non-aerated conditions.

The byproducts produced during ethanol production cause an inhibitory effect on ethanol yield. Under the aerated environment, the cell count viability declined much slower when the cell count viability was compared with the non-aerated environment at all sugar concentration during fermentation. Whereas under a non-aerated and aerated environment, the viability of cells decreased after 51 hours of operation. This reduced viability of yeast cells clearly indicated for more aeration to overcome the osmotic stress at the initial stage of reactor operation, furthermore at the end of the fermentation process more aeration was needed to overcome the ethanol-induced oxidative stress. This behavior of fermentation using aeration and the non-aeration environment was in good agreement with the results gathered by the researcher (Cot, Loret, François, & Benbadis, 2007). while the research done by the researcher observed that the cell viability and cell count increased with an increase in aeration level (Maemura, Morimura, & Kida, 1998). The cell count in fermenter became maximum after 25 hours of fermenter operation and the viability and cell count was highly dependent on the aeration level. The dissolved oxygen level increased when the aeration level was enhanced, while at higher Brix the cell count was decreased at low aeration. Various researchers also observed that when ethanol production within the fermenter increased the count started to decrease. Moreover, the production of ethanol in this study was highest at lower air supply and this trend of ethanol production was in line with the findings achieved by (Seo, Kim, Lee, & Jung, 2009). At the later stage of fermentation, the growth of cell and ethanol yield decreases as the concentration of ethanol reaches 100g/L. However the yield of ethanol was higher under increased aeration atmosphere, a similar trend was observed by the researcher(Alfenore et al., 2004). Moreover in this research study production of ethanol under very high gravity along with air supply produces higher ethanol and minimum by-products and these results are in line with the research done by(Arshad et al., 2017)

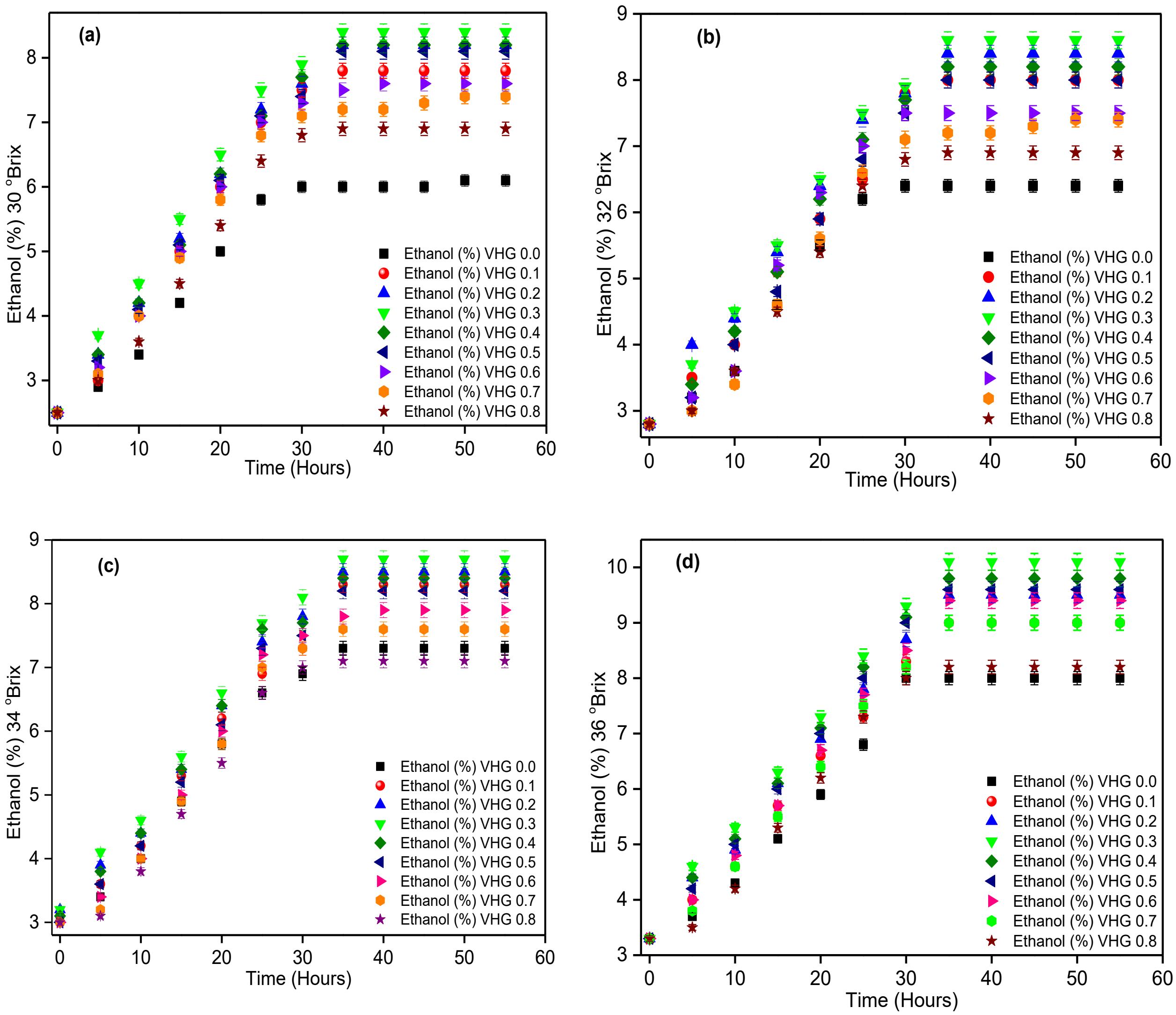

Fig. 3(a-d) Ethanol production at 30, 32, 34 and 36°Brix.

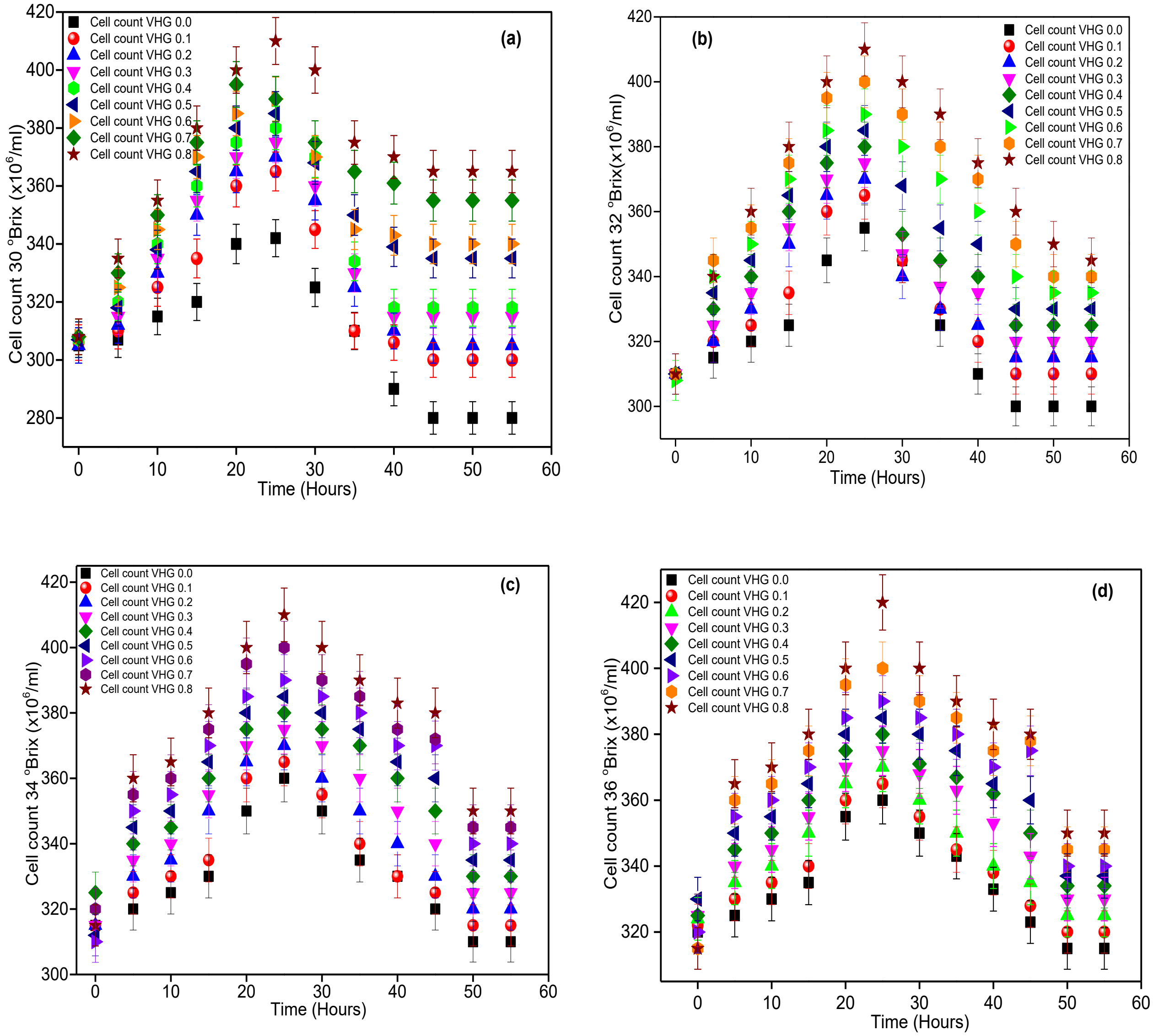

Fig. 4(a-d) Cell count at 30, 32, 34 and 36 °Brix.

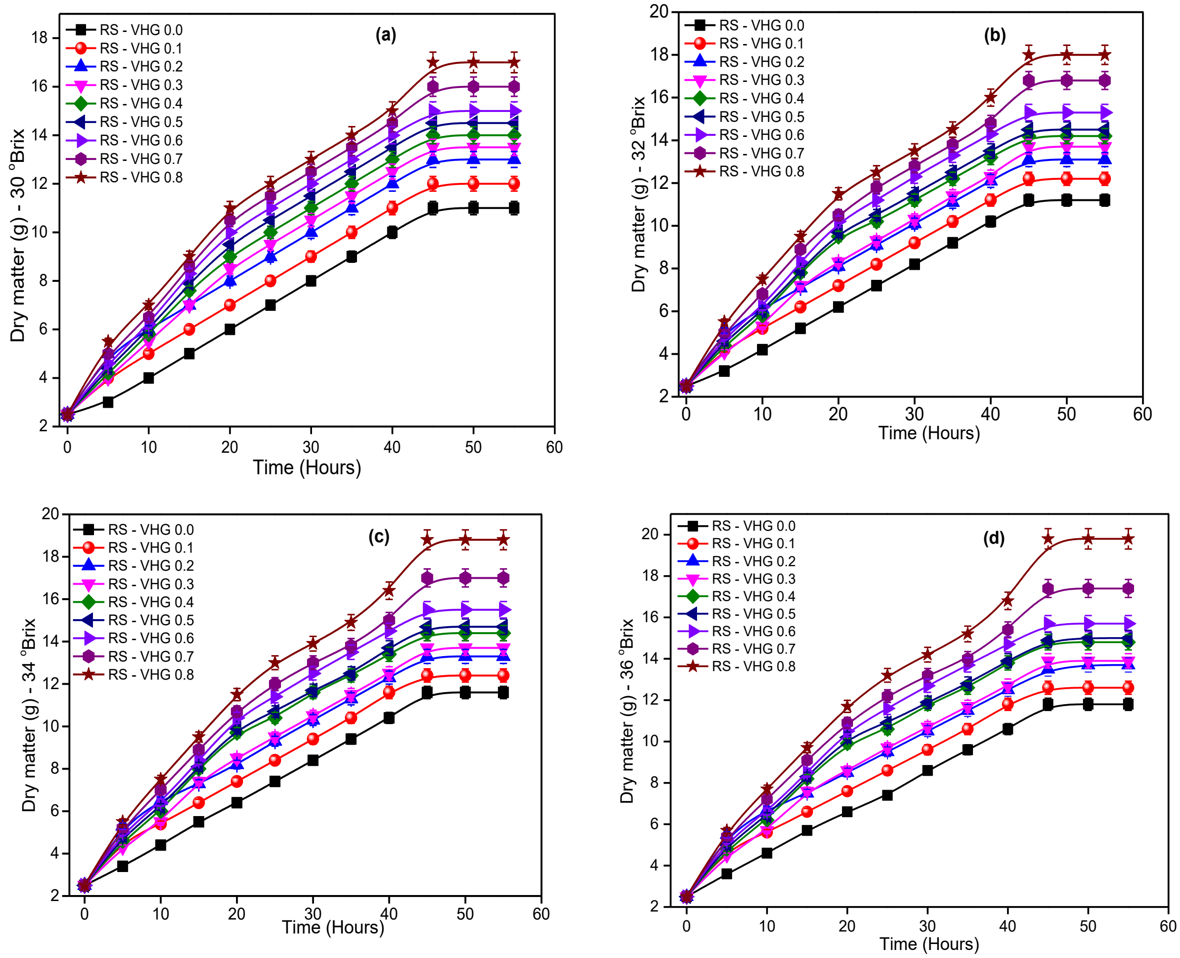

Fig.5 (a-d) Dry matter at varying time.

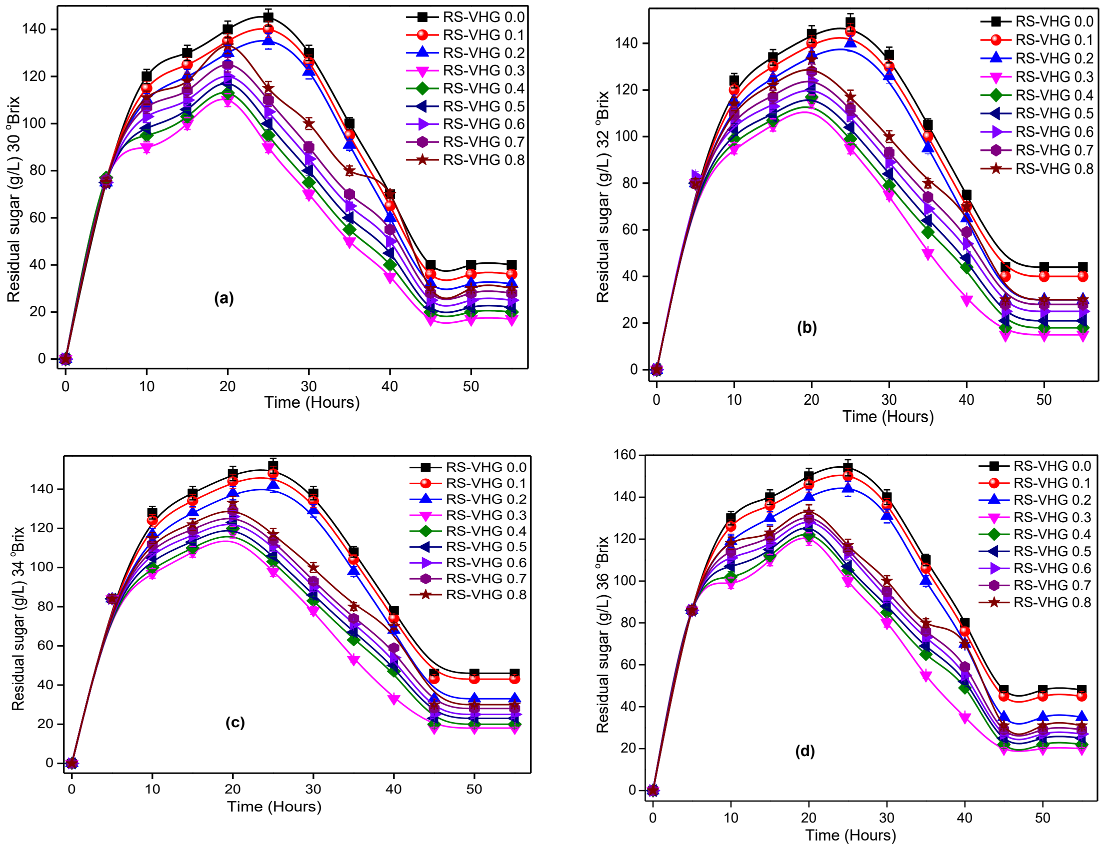

Fig. 6(a-d) Residual sugar at 30, 32, 34 and 36 °Brix.

Fig. 7 shows the effect of pH on ethanol yield during fermentation. The behavior of fermentation yield was investigated at varying pH levels from 3.5 to 8.5. The highest yield of ethanol from fermentation was achieved at a pH level of 5.5 was achieved 10.5. More the acidic nature of fermentation was favorable for enhanced activity of Saccharomyces cerevisiae yeast. The invertase enzyme converted sucrose to fermentable sugars efficiently under acidic conditions. The fermentation behavior was very low at neutral pH of 7. While under basic medium there was no conversion of sugars into ethanol yield.

Fig. 7 shows the effect of temperature on fermentation yield. As different temperature ranges were investigated for achieving maximum ethanol yield. The optimum fermentation yield was achieved at a temperature of 30°C. The alcoholic fermentation process stimulates and accelerates at 30°C and ethanol production reached 10.5, while at a temperature below 30°C, the fermentation reaction was slower producing low alcohol. Moreover, above 30°C temperature, the fermentation efficiency was reduced, and above 36°C fermentation, cells started to die. While above 41°C most of the cells died and the fermentation reaction was stopped.

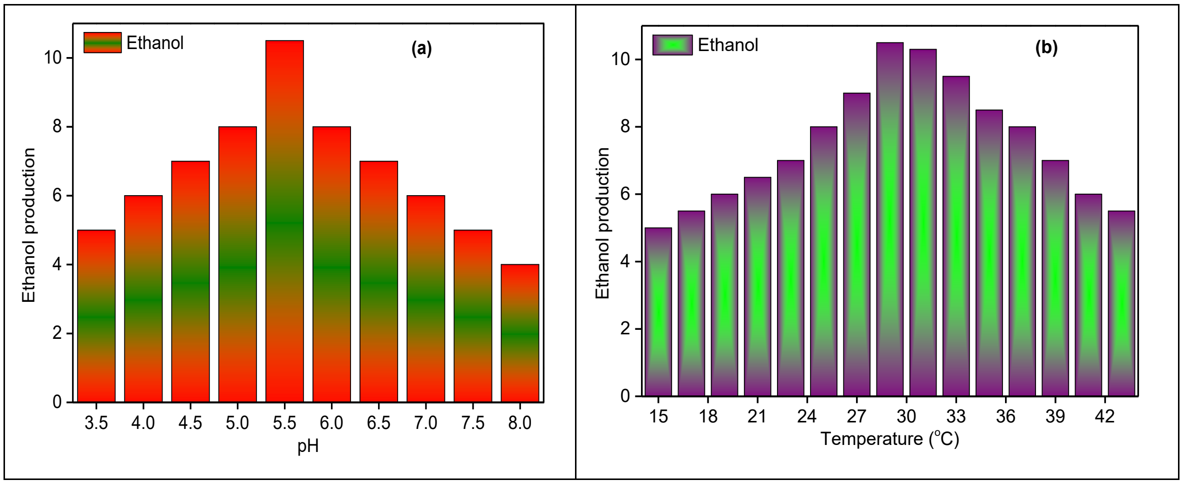

Fig. 7(a-b) Effect of pH and temperature on fermentation yield.

Fig. 8 represents the behavior of fermentative behavior of the yeast cells at different doses of penicillin. From Fig. 8 it was investigated that the application of penicillin did not directly affect the fermentative activity of the yeast cells and acts only as an inhibitor of contaminants .

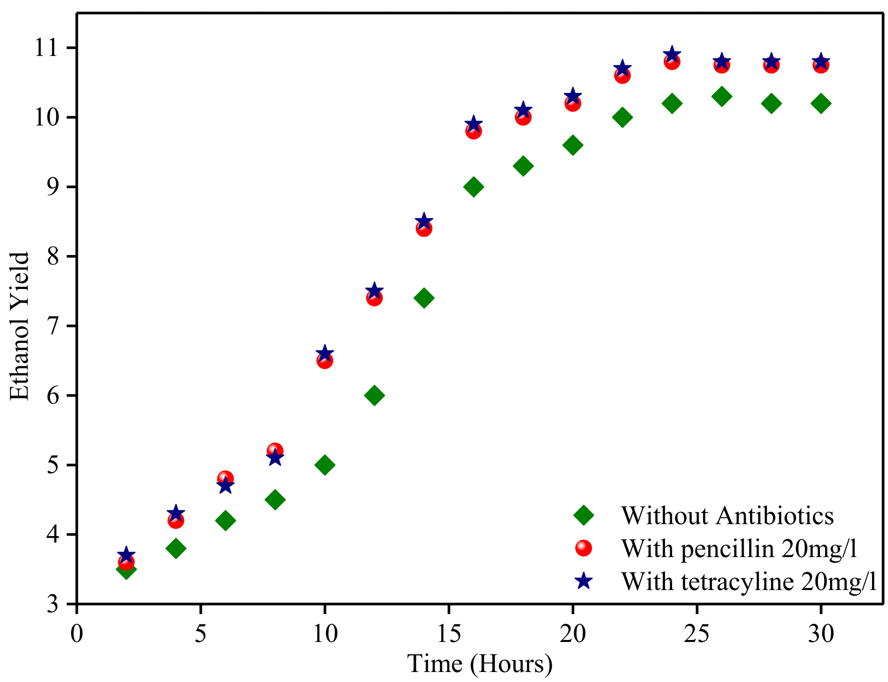

Fig. 8 effect of penicillin and tetracycline on alcoholic concentration.

Fig. 8 The effect of three doses of tetracycline at different intervals of time was done to measure the effect of tetracycline on fermentative efficiency of yeast. The first dose of tetracycline was added at the start of the fermentation to avoid the adaptation of foreign microorganisms while the second dose was introduced after five hours of fermentation when fermentation rate started to increase. Moreover, the third dose was introduced after 22 hours of fermentation. Then the comparison of several doses with single dose was compared during fermentation gave similar results. The production of ethanol increased with the addition of penicillin and tetracycline as compared with the production of alcohol with the application of penicillin and tetracycline.

3. CONCLUSION

The residual sugar was increased up to 30 hours of fermentation and then started to decrease up to 60 hours of operation. With the increase in sugar level the viable cell count was increased up to 497 million/Ml. The °Brix level significantly affected ethanol production, the increase in ethanol production and decrease in residual sugar is good indication of fermentation. The optimum ethanol yield was achieved 10.9 v/v at 38°Brix with 0.25vvm aeration rate. At the aeration rate of 0.25, 0.35 and 0.45vvm was 17.6, 23.7 and 20.9 g/L respectively. Moreover, in non-aerated environment the residual sugar was observed 58.4g/L. Under non aeration conditions the viability of yeast cells declined 25% at 34°Brix. The residual sugar was very high as compared with aerated conditions. Moreover, ethanol production was also improved by 18% by maintain 0.2vvm aeration conditions in fermenter. The highest yield of ethanol from fermentation was achieved at a pH level of 5.5 was achieved 10.5. More the acidic nature of fermentation was favorable for enhanced activity of *Saccharomyces cerevisiae* yeast. The optimum fermentation yield was achieved at a temperature of 30ºC. The alcoholic fermentation process stimulates and accelerates at 30ºC and ethanol production reached 10.5, while at a temperature below 30ºC, the fermentation reaction was slower producing low alcohol. The production of ethanol increased with the addition of penicillin and tetracycline as compared with the production of alcohol with the application of penicillin and tetracycline.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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REFERENCES

Alfenore, S., Cameleyre, X., Benbadis, L., Bideaux, C., Uribelarrea, J.-L., Goma, G., . . . Guillouet, S. (2004). Aeration strategy: a need for very high ethanol performance in Saccharomyces cerevisiae fed-batch process. *Applied microbiology and biotechnology, 63*(5), 537-542.

Ali, F., Jiang, Y., & Khan, K. (2017, 10-13 Dec. 2017). Feasibility analysis of renewable based hybrid energy system for the remote community in Pakistan. Paper presented at the 2017 IEEE International Conference on Industrial Engineering and Engineering Management (IEEM).

Amamra, S., Meghriche, K., Cherifi, A., & Francois, B. (2017). Multilevel Inverter Topology for Renewable Energy Grid Integration. *IEEE Transactions on Industrial Electronics, 64*(11), 8855-8866. doi:10.1109/TIE.2016.2645887

Arshad, M., Hussain, T., Iqbal, M., & Abbas, M. (2017). Enhanced ethanol production at commercial scale from molasses using high gravity technology by mutant S. cerevisiae. *brazilian journal of microbiology, 48*, 403-409.

Bibi, R., Ahmad, Z., Imran, M., Hussain, S., Ditta, A., Mahmood, S., & Khalid, A. (2017). Algal bioethanol production technology: A trend towards sustainable development. *Renewable and Sustainable Energy Reviews, 71*, 976-985. doi:https://doi.org/10.1016/j.rser.2016.12.126

Boonchuay, P., Techapun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., Watanabe, M., . . . Chaiyaso, T. (2018). An integrated process for xylooligosaccharide and bioethanol production from corncob. *Bioresource Technology, 256*, 399-407. doi:https://doi.org/10.1016/j.biortech.2018.02.004

Byadgi, S. A., & Kalburgi, P. B. (2016). Production of Bioethanol from Waste Newspaper. *Procedia Environmental Sciences, 35*, 555-562. doi:https://doi.org/10.1016/j.proenv.2016.07.040

Chao, B., Liu, R., Zhang, X., Zhang, X., & Tan, T. (2017). Tannin extraction pretreatment and very high gravity fermentation of acorn starch for bioethanol production. *Bioresource Technology, 241*, 900-907. doi:https://doi.org/10.1016/j.biortech.2017.06.026

Cot, M., Loret, M.-O., François, J., & Benbadis, L. (2007). Physiological behaviour of Saccharomyces cerevisiae in aerated fed-batch fermentation for high level production of bioethanol. *FEMS Yeast Research, 7*(1), 22-32. doi:10.1111/j.1567-1364.2006.00152.x

de Carvalho, A. L., Antunes, C. H., & Freire, F. (2016). Economic-energy-environment analysis of prospective sugarcane bioethanol production in Brazil. *Applied Energy, 181*, 514-526. doi:https://doi.org/10.1016/j.apenergy.2016.07.122

Denholm, P., Brinkman, G., & Mai, T. (2018). How low can you go? The importance of quantifying minimum generation levels for renewable integration. *Energy Policy, 115*, 249-257.

e Silva, J. O. V., Almeida, M. F., da Conceição Alvim-Ferraz, M., & Dias, J. M. (2018). Integrated production of biodiesel and bioethanol from sweet potato. *Renewable Energy, 124*, 114-120.

Kamran, M., Fazal, M. R., & Mudassar, M. (2020). Towards empowerment of the renewable energy sector in Pakistan for sustainable energy evolution: SWOT analysis. *Renewable Energy, 146*, 543-558. doi:https://doi.org/10.1016/j.renene.2019.06.165

Liu, Y.-M., Hu, C.-Y., Shen, J.-D., Wu, S.-H., Li, Y.-C., & Yi, L.-T. (2017). Elevation of synaptic protein is associated with the antidepressant-like effects of ferulic acid in a chronic model of depression. *Physiology & Behavior, 169*, 184-188. doi:https://doi.org/10.1016/j.physbeh.2016.12.003

Madhania, S., Muharam, Y., Winardi, S., & Purwanto, W. W. (2019). Mechanism of molasses–water mixing behavior in bioethanol fermenter. Experiments and CFD modeling. *Energy Reports, 5*, 454-461.

Maemura, H., Morimura, S., & Kida, K. (1998). Effects of aeration during the cultivation of pitching yeast on its characteristics during the subsequent fermentation of wort. *Journal of the Institute of Brewing, 104*(4), 207-211.

Mohd Azhar, S. H., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Mohd Faik, A. A., & Rodrigues, K. F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports, 10*, 52-61. doi:https://doi.org/10.1016/j.bbrep.2017.03.003

Rahimi, M. (2005). The effect of impellers layout on mixing time in a large-scale crude oil storage tank. *Journal of Petroleum Science and Engineering, 46*(3), 161-170. doi:https://doi.org/10.1016/j.petrol.2004.12.002

Saez-de-Ibarra, A., Martinez-Laserna, E., Stroe, D., Swierczynski, M., & Rodriguez, P. (2016). Sizing Study of Second Life Li-ion Batteries for Enhancing Renewable Energy Grid Integration. *IEEE*

Transactions on Industry Applications, 52(6), 4999-5008. doi:10.1109/TIA.2016.2593425

Seo, H.-B., Kim, S. S., Lee, H.-Y., & Jung, K.-H. (2009). High-level production of ethanol during fed-batch ethanol fermentation with a controlled aeration rate and non-sterile glucose powder feeding of Saccharomyces cerevisiae. *Biotechnology and Bioprocess Engineering, 14*(5), 591. doi:10.1007/s12257-008-0274-2

Sossa-Echeverria, J., & Taghipour, F. (2015). Computational simulation of mixing flow of shear thinning non-Newtonian fluids with various impellers in a stirred tank. *Chemical Engineering and Processing: Process Intensification, 93*, 66-78. doi:https://doi.org/10.1016/j.cep.2015.04.009

Tgarguifa, A., Abderafi, S., & Bounahmidi, T. (2017). Modeling and optimization of distillation to produce bioethanol. *Energy Procedia, 139*, 43-48. doi:https://doi.org/10.1016/j.egypro.2017.11.170

Tomás-Pejó, E., Oliva, J. M., González, A., Ballesteros, I., & Ballesteros, M. (2009). Bioethanol production from wheat straw by the thermotolerant yeast Kluyveromyces marxianus CECT 10875 in a simultaneous saccharification and fermentation fed-batch process. *Fuel, 88*(11), 2142-2147. doi:https://doi.org/10.1016/j.fuel.2009.01.014

Wakeel, M., Chen, B., & Jahangir, S. (2016). Overview of Energy Portfolio in Pakistan. *Energy Procedia, 88*, 71-75. doi:https://doi.org/10.1016/j.egypro.2016.06.024

Wang, L., Sharifzadeh, M., Templer, R., & Murphy, R. J. (2013). Bioethanol production from various waste papers: economic feasibility and sensitivity analysis. *Applied Energy, 111*, 1172-1182.