Ethanol production from food waste

Environment, Pollution

Ethanol Production From Food Waste A PROJECT REPORT Submitted in partial fulfillment of the requirements for the Award of the Degree of Bachelor of Technology (Biotechnology) Under the Guidance of Dr. S. M. Bhatt (Associate Professor) Department of Biosciences By Abhishek Agarwal Registration No. 10809065 Roll No. RB18B2A07 Department of Biotechnology Engineering Lovely Professional University Phagwara —144401 November 2011 CERTIFICATE This is to certify that Abhishek Agarwal bearing Registration no. 10809065 has completed minor project titled, “ Ethanol Production from Food Waste" under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of the dissertation has ever been submitted for any other degree at any University. The dissertation is fit for submission and the partial fulfillment of the conditions for the award of degree of Bachelor of Technology. Date Dr. S. M. Bhatt Assistant Professor Biometric Id 14722 Lovely School Of Bio Sciences Lovely Professional University Phagwara, Punjab. DECLARATION I, Abhishek Agarwal , student of Btech Biotech+M. B. A., under Department of Biotechnology Engineering of Lovely Professional University, Punjab, hereby declare that all the information furnished in this minor project report is based on my own intensive research and is genuine. This report does not, to the best of my knowledge, contain part of my work which has been submitted for the award of my degree either of this university or any other university without proper citation. Date : Abhishek Agarwal 10809065 ACKNOWLEDGEMENT The key elements concentration, dedication, hard work and application are not the only essential factors for achieving the desired goals but also guidance, assistance and co-operation

of people is necessary. I would like to express my deep and sincere gratitude to my supervisor Dr. S. M. Bhatt Associate Professor. His wide knowledge and logical way of thinking have been of great value for me. His understanding and personal guidance have provided a good basis for the present report. Especially the strict and extensive comments and many discussions and the interactions with Dr. Bhatt had a direct impact on the final form and quality of this report. I also thank to all the faculty and staff members of Department of biotechnology Engineering, Lovely Professional University, Phagwara, for their co-operation and support throughout the course work and also during my entire study period. I would like to make special acknowledge to some of my friends, who have been always there for me, listening to me, rejoicing, complaining and pondering my way throughout my B. Tech. study. I was very fortunate to have unconditional support from my family throughout this time. They have elucidated me the meaning of life, love and living. My loving thanks to my mother who has always kept my morals high through tough times. (Abhishek Agarwal) INTRODUCTION Growth of population, increasing urbanization, rising standards of living due to technological innovations have contributed to an increase both in the quantity and variety of solid wastes generated by industrial, mining, domestic and agricultural activities. Total commercial and industrial (C&I) waste arisings in 2009 are estimated to be 55. 8 million tonnes. The industrial sector accounts for 20. 5 million tonnes, or 37%, with the commercial sector accounting for 35. 3 million tonnes, or 63%.[1] Food waste or food loss is food that is discarded or lost uneaten. As of 2011, 1. 3 billion tons of food, about one third of the global food production, is lost or wasted annually. Loss and wastage occurs on all steps

in the food supply chain. In low-income countries most loss occurs during production, while in developed countries much food — about 100 kilograms (220 lb) per person and year — is wasted at the consumption stage.[2] About 90 billion tones solid wastes are expected to be generated annually by the year 2025. Annually, Asia alone generates 4. 4 billion tones of solid wastes and municipal solid waste comprises 790 million tones of which about 48 million tones are generated in India. By the year 2047, municipal solid waste generation in India, is expected to reach 300 million tones and land requirement for disposal of this waste would be 169. 6 km.[3]. Solid waste generation from organic sources include municipal and urban wastes, animal wastes, farming wastes, horticulture wastes, domestic refuses and agro-industrial wastes. India is one of the richest countries in agricultural resources. Agricultural wastes are the byproducts of various agricultural activities such as crop production, crop harvest, saw milling, agro-industrial processing and others. The major quantity of wastes generated from agricultural resources are sugarcane baggase, paddy and wheat straw and husk, wastes of vegetables, food products, jute fiber, groundnut shell, coconut husk and cotton stalk etc. The main objective of waste management system is to maximize economic benefits and at the same time protection of environment. However, it is envisaged that the total solid wastes from municipal, agricultural, non-hazardous and hazardous wastes generated from different industrial processes in India seem to be even higher than the reported data. Already accumulated solid waste and their increasing annual production are a major source of pollution. Due to the environmental degradation, energy consumption and financial constraints, various

organizations in India and abroad, apart from the regulatory frame work of United States Environmental Protection Agency, has recommended various quantitative guidelines for generation, treatment, transport, handling, disposal and recycling of non-hazardous and hazardous wastes. In developing countries, there is a different approach to dealing with organic waste. In fact, the word 'waste' is often an inappropriate term for organic matter, which is often put to good use.[4] The economies of most developing countries dictates that materials and resources must be used to their full potential, and this has propagated a culture of reuse, repair and recycling. In many developing countries there exists a whole sector of recyclers, scavengers and collectors, whose business is to salvage 'waste' material and reclaim it for further use. Humans have been producing ethanol for thousands years. The very first time, ethanol existed only in alcoholic drinks. After some purification methods were established, the usage of ethanol highly extended. Ethanol has high latent heat of vaporization, high octane number and emission of toxic compounds on its combustion is low. It is argued that it has lower burning value than gasoline but this will be compensated by high latent heat of vaporisation which is nearly double than that of gasoline. Ethanol is more eco-friendly as its combustion releases carbon dioxide which is far less harmful than carbon monoxide released by the combustion of gasoline; it can also reduce our dependence on fossil fuels as most of the features of gasoline are similar to that of ethanol.[5][6][7] According to the time flow, the area of ethanol has been extending dramatically. The current increase in the gas price and interest in environmental problems, ethanol becomes highly attractive again. In the

present study food wastes were examined and used for production of ethanol by using alpha-amylase also produced by food wastes and selection of thermostable yeasts. LITERATURE REVIEW Waste according to United Nations[2] Food loss measures the decrease in edible food mass (excluding inedible parts and seed) " throughout the part of the supply chain that specifically leads to edible food for human consumption", that is, loss at the production, postharvest and processing stages. This definition of loss includes biomass originally meant for human consumption but eventually used for some other purpose, such as fuel or animal feed. Food waste is food loss occurring during the retail and final consumption stages due to the behavior of retailers and consumers- that is, the throwing away of food Waste according to European Union[8] The EU defines waste as an object the holder discards, intends to discard or is required to discard is waste under the Waste Framework Directive (European Directive 75/442/EC as amended). “ Once a substance or object has become waste, it will remain waste until it has been fully recovered and no longer poses a potential threat to the environment or to human health". Cellulosic agro-waste Cellulosic biomass constitutes a huge and renewable resource that can be converted to compost and fuel feedstocks. More efficient means for conversion of agricultural and forest waste are sought so that useful biomass-derived products can not only compete with or eventually replace petroleum based products but also supplement and complement the use of petroleum based fuels as additives to promote more efficient burning and lower emissions. Using these cellulosic resources efficiently can thus reduce the disposal problems and pollution resulting from accumulation of these wastes (Edwin,

2001). Cellulose is a major component of the cell wall of plants and the most abundant and renewable carbohydrate; therefore, practical and cost-effective processes for bioethanol production from cellulose are highly desired. Cellulosic biomass is a complex mixture of carbohydrate polymers from plant cell walls known as cellulose and hemicellulose, plus lignin and a smaller amount of other compounds generally known as extractives. For the cellulosic biomass to degrade we need to convert the hemicelluloses to monomeric sugars by the process of pretreatment Pre-treatment increases the crystallinity of cellulose, while removing lignin and other inhibitors, thereby enabling its enzymatic degradation. In addition, pretreatment may increase the surface area of the cellulose thereby enhancing its reactivity with the enzyme and thus its transformation. During the pretreatment process, degradation compounds of pentoses and hexoses primarily furfural and 5-hydroxymethyl furfural (5-HMF) are formed. There may also be acid from the pretreatment if acid hydrolysis has been performed. These components are toxic and inhibit the subsequent enzymatic and fermentative processes. Therefore, they must be removed or neutralised prior to the fermentation; otherwise, larger amounts of fermenting microorganisms need to be applied in fermentation[9-14]. A simple flowchart will illustrate the breakdown of hemicelluloses to sugars. Figure 1; Process diagram for degradation of biomass Now in this process two enzymes are involved mainly which are responsible for the degradation of hemicelluloses to glucose these are endoenzymealpha-amylase and exoenzymeglucoamylase.[18] In the first step pretreatment of the wastes leads to reduction of size of the chain. Now alpha-amylase acts on the

attacks the alpha-1, 4 linkages of starch. Figure 2; Structure of starch [image courtesy Davidson College Home page; My Favorite Protein: Î±-amylase] Now when action of alpha-amylase is completed and dextrins are produced then glucoamylase acts on the dextrin molecules to removes one glucose molecule from the dextrins Figure 3; Structure of Dextrin Figure 4. The hydrolysis of starch to glucose catalyzed by Î±-amylase [image courtesy Davidson College Home page; My Favorite Protein: Î±-amylase] Figure 5; A simplified schematic representation of the process involved in complete enzymatic hydrolysis of a cellulose biomass.[16] Hydrolysis Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials. In this method, chipped biomass is treated with high-pressure saturated steam and then pressure is swiftly reduced, which makes the materials undergo an explosive decompression, this causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis.[17] Acid hydrolysis Concentrated acids such as H2SO4 and HCl have been used to treat lignocellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible. Dilute acid hydrolysis has been successfully developed for pretreatment of lignincellulosic materials. The dilute sulphuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis.[18]At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature

in dilute acid, treatment is favorable for cellulose hydrolysis [17]. Now these monomeric sugars are fermented by the action of natural or genetically engineered yeasts such as S. cerevisae and these convert the sugars into ethanol which can be explained by a simple chemical reaction: C6H12O6 (glucose) –> 2 CH3CH2OH (ethanol)+ 2 CO2 (carbon dioxide) (anaerobic process)[15] This ethanol produced is considered biologically safe because Ethanol represents closed carbon dioxide cycle because after burning of ethanol, the released carbon dioxide is recycled back into plant material because plants use CO2 to synthesize cellulose during photosynthesis cycle. Ethanol production process only uses energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source. In addition, the toxicity of the exhaust emissions from ethanol is lower than that of petroleum sources[5-7]. Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect. Ethanol is then recovered by distillation or filteration. Distillation Distillation is a separation process for a mixture of liquids or oils. It relies on differences in the boiling points of the component liquids to be separated. Distillation was one of the earliest separation techniques used by alchemists and pharmacists. And, generally, distillation, along with chromatography and filtration, is still considered to be a key method of separating and purifying substances. In our distillation, Ethyl Alcohol has a boiling point of 78. 5oC and that of Water is 100oC.[19] Objectives 1. Amylase Production by Sprouts + Rice 2. Ethanol Production by Baker’s Yeast in Cane Molasses 3. Ethanol Production by Baker’s Yeast in Digested Rice 4. Ethanol Estimation by Potassium Dichromate Assay. 5.

Recovery of Ethanol Materials and methods Cane Molasses Cane molasses was collected from outskirts of Lovely Professional University in a conical flask in sufficient amount around 500ml, then a pretreatment process for the size reduction was done in which the sample was given a mild acid treatment with dilute sulphuric acid all the cane molasses were soaked in acid for one hour and then was washed with distilled water four times this led to reduction of size and softening of the cane upto some extent and then the sample was incubated at 37°C inside the incubator for ten days. Rice Wasted Cooked rice around 200 grams was collected from the food court of Lovely Professional University and were stored in a conical flask at room temperature for two days. Sprouts Uncooked white sprouts were collected around 100 grams and were dipped in sufficient amount of water and left for three days so that sprouting can be achieved. Then when the sprouting was observed these were grinded in a mortar pastel along with sufficient amount of distilled water and the paste was then centrifuged at 5000 rpm for 10 minutes in the centrifuge tubes. The supernatant was collected and 70 ml was inoculated in the already stored cooked rice and then the mixture was incubated at 25o C inside the incubator for ten days. Baker’s Yeast Readily available yeast which has application in the baking industry for fermentation was collected in small quantity 20 grams from a local Bakery at Model Town area of Jalandhar and a solution was prepared by dissolving 20 grams of Baker’s Yeast in 200 ml of autoclaved water and was stored inside the incubator at 37oC till further use. Fermentation Process Then after ten days of incubation for the sprout-rice mixture physical examination was done for digestion of rice and now there was no solid part of rice present in the

mixture so this meant that digestion of rice by the action of bacteria, yeasts and amylases has taken place, then aliquots of 2 ml was done from the mixture and was inoculated inside the already incubated cane molasses so that further digestion of starch present in the molasses to monomer forms of convertible sugars was done. Then the already prepared solution of Baker’s Yeast was added 3 ml to the cane molasses and sprouts mixture and was mixed well using a glass rod and the mixture was again incubated for fifteen days at 37°C inside the incubator. Then in the digested rice mixture also Baker’s Yeast was added 5 ml and was well mixed using glass rod and was incubated at 25oC for fifteen days inside the incubator. During the incubation period some physical indicators such as:- bubbles, taste and hissing noise were examined in both the incubated flasks of cane molasses and digested rice and then small aliquots were taken from the mash for ethanol identification and estimation. Preparation of Standard Ethanol Curve Potassium dichromate method was used for the estimation of ethanol in the samples prepared. But in order to use this method first we need to determine the standard curve of the absorbance v/s concentration for the standard ethanol solutions. Material 1. Absolute Ethanol 10 ml, 2. Distilled Water 200 ml, 3. Test tubes 10 4. Spectrophotometer unit. Method a. Different concentrations of ethanol ranging between 0. 5%-1% of ethanol were made by mixing proportional amount of absolute ethanol in each tube having 10 ml distilled water. b. Distilled water was used as Blank solution as reference solution for spectrophotometer c. Then absorbance was recorded for all the solutions by following the operating protocol at 600 nm. d. Then a graph was plotted between Absorbance at 600 nm on y-axis and

concentration of ethanol on x-axis. Potassium Dichromate Method for Ethanol Estimation Material 1. Potassium dichromate (K2Cr2O7) 2. Distilled water 3. Concentrated sulphuric acid. Method a. Potassium dichromate (K2Cr2O7) assay was prepared by dissolving 8. 44 gm of K2Cr2O7 in 100 ml distilled water and 81. 25 ml concentrated sulphuric acid, cooled and then made total volume to 250 ml with distilled water. b. Different dilutions of cane molasses solution were prepared by mixing stock cane molasses solution (5 %) and diluted to 10 ml with distilled water in the test tube. c. To this 2 ml of K2Cr2O7 (1N) solution already prepared was added & mixed. d. This solution was boiled for exactly 10 min in a vigorously boiling water bath. e. The presence of ethanol will lead to a change in color to green from orange. f. Then absorbance was taken at 600 nm in spectrophotometer after 10 minutes. g. Then the optical densities of the samples were compared with that of standard ethanol OD value concentration of ethanol were determined in both the samples. Filtration of Ethanol When the results of ethanol estimation were found positive and there was production of ethanol in the mash then filtration and distillation of the mash was initiated. First of all the mash was filtered by using muslin cloth, the cloth was fixed with a glass funnel over a conical flask and then slowly the mash of cane molasses was poured over the funnel and then left for filtration. The solid and undigested part is removed and clear liquid was collected. This liquid was once again filtered by using filter paper so that more clear liquid is obtained. The amount of filtrate produced was measured. for both the samples using a measuring cylinder. Distillation of Ethanol Then after this distillation of ethanol was performed in which absolute ethanol was collected. The clear

liquid collected from the mash of cane molasses was added to a Distilling Pot where it is heated to the temperature of 75oC. As lower boiling components will preferentially vaporize first so the ethanol having boiling point of 78. 5oC will start boiling up and forming vapor before any water then this vapor passed into a Distilling Head and then into a Condenser. Within the Condenser the vapor is cooled and it liquefies. The resulting liquid is then collected in a Receiving Flask and was stored at room temperature in a round bottom flask. Final volumes of the distilled liquid were recorded. Results The mash was examined everyday during the incubation period and physical factors were examined. After the incubation period of twelve days for the cane molasses sample the three physical factors which were examined gave a result :- 1. There is no more bubbles coming to the surface. 2. There is no more hissing noise inside the vessel. 3. The mash does not taste sweet anymore. These results indicated that the fermentation process has stopped in the vessel and yeasts are not functional at that time. After a incubation period of fourteen days these factors were seen in the digested rice mixture. Standard Ethanol Curve Graph was plotted between standard ethanol concentrations and absorbance the graph was nearly a straight line with a R2 value= 0. 985. Here distilled water was used as Blank Solution for the reference. Calculations were done using the formulae:- Mass% of A in B= vol% (A in B)* density ratio of A/B Here A is ethanol and B is distilled water. Density ratio is 0. 8. So on putting the values in the formulae we get these values 0. 5%*0. 8= 0. 4 0. 6%*0. 8= 0. 48 0. 7%*0. 8= 0. 56 0. 8%*0. 8= 0. 64 0. 9%*0. 8= 0. 72 1%*0. 8= 0. 8 Observation table for standard ethanol Table 1; standard table for absorbance with concentration Curve

Concentration of ethanol in mg Graph 1; Curve between absorbance at 600 nm v/s concentration of ethanol in mg For digested rice serial dilutions with a 0. 5 ml sample in 9. 5 ml water and 2 ml of K2Cr2O7 solution and then doing serial dilutions dilutions | OD value | a | 0. 823 | b | 0. 463 | c | 0. 158 | d | 0. 041 | For cane molasses serial dilutions with a 0. 5 ml sample in 9. 5 ml water and 2 ml of K2Cr2O7 solution and then doing serial dilutions Now these data's are interpreted with the standard OD values to estimate ethanol concentration in the samples. For digested rice From the equation y= 1. 039 x we know the value of y in all the cases and need to determine the value of x so formulae is modified as:- X= y/1. 039 Dilution a is 0. 5 ml sample in 9. 5 ml of distilled water (5% solution.) Dilution b is 1 ml of dilution a in 9 ml of water Dilution c is 1 ml of dilution b in 9 ml of water Dilution d is 1 ml of dilution c in 9 ml of water final average conc in mg= (conc in a + conc in b = conc in c + conc in d)/4 0. 357 mg Ethanol production in cane molasses after addition of sprout+ baker's yeast X= y/1. 039 Dilution a is 0. 5 ml sample in 9. 5 ml of distilled water (5% solution.) Dilution b is 1 ml of dilution a in 9 ml of water Dilution c is 1 ml of dilution b in 9 ml of water Dilution d is 1 ml of dilution c in 9 ml of water final average conc in mg= (conc in a + conc in b = conc in c + conc in d)/4 = 0. 271 mg Filtrate collected after filtration For waste rice= 60ml For cane molasses= 115ml Conclusion It is concluded that large amount of ethanol can be produced by using starch rich waste materials by the action of several enzymes and microbes and this process can be made more efficient by providing the mash with controlled environment and also by improving the quality of our raw material and selecting the waste material that has greater starch content so that it can be

further digested to form sugars and then ethanol is produced. References 1. Statistics available online http://www. defra. gov. uk/news/files/2010/11/1011stats. pdf 2. Gustavson, Jenny; Cederberg, Christel; Sonesson, Ulf; van Otterdijk, Robert; Meybeck, Alexandre (2011). Global Food Losses and Food Waste; p 4 3. (Aziz S., Sarkanen K., 1989. Organosolv pulping — a review. Tappi. J. 72, 169-175 4. Morris, Robert F.; United States National Research Council (1978). Postharvest food losses in developing countries. National Academy of Sciences. Retrieved 2009-08-24. 5. Atkins, Peter (2010). Atkins' Physical Chemistry. Oxford University Press. pp. 913-947. 6. Lange's Handbook of Chemistry 10th ed, pp 1525-1528 7. Appendix B, Transportation Energy Data Book from the Center for Transportation Analysis of the Oak Ridge National Laboratory 8. " The Definition of Waste, Summary of European Court of Justice Judgments" Defra. Updated 2009. Retrieved 2009-08-20 9. del Campo, I. Alegr´Ä±a, M. Zazpe, M. Echeverr´Ä±a, I. Echeverr´Ä±a 2006 Diluted acid hydrolysis pretreatment of agri-food wastes for bioethanol production 10. Cysewski G. R., Wilke C. R., (1976). Utilization of cellulosic materials through enzymatic hydrolysis and fermentation of hydrolysate to ethanol and single cell protein. Biotechnol. Bioeng. 18: 1297— 1313. 11. Dale B. E., Moreira M. J., 1982. A freeze explosion technique for increasing cellulose hydrolysis. Biotechnol. Bioeng. Symp. 12, 31-43. 12. Taherzadeh MJ, Karimi K (2007). " Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review" 13. Shawn O'Conner, Mark Eiteman; Ethanol Production from Food Residues 14. Seungdo Kim, Bruce E.; 31 July 2003; Global potential bioethanol production from wasted crops and crop residues 15. Morais PB, Rosa CA, Linardi VR,

Carazza F, Nonato EA (1996). " Production of fuel alcohol by Saccharomyces strains from tropical habitats". Biotechnology Letters 18 (11): 1351. doi: 10. 1007/BF00129969 16. Fillingham IJ, Kroon PA, Willaimson G, Gilbert HJ & Hazlewood GP (1999) A modular cinnamoyl ester hydrolase from the anaerobic fungus Piromyces equi acts synergistically with xylanase and is part of a multiprotein cellulose-binding cellulase-hemicellulase complex. Biochem. J. 343: 215—224 17. McMillan J. D., 1994. Pretreatment of lignocellulosic biomass. In: Himmel M. E., Baker J. O., Overend R. P. (Eds.), Enzymatic Conversion of Biomass for Fuels Production. American Chemical Society, Washington DC, pp. 292-324. Goel AK (2002). A sugar industry perspective. Ethanol: Winrock International India, New Delhi, August 2002. p. 5. 18. Wheals, A. E.; Basoo, L. C.; Alves, D. M. G.; Amorim, H. V., Fuel ethanol after 25 years. Tibtech, 17 (1999 19. The Manual for the Home and Farm Production of Alcohol Fuel by S. W. Mathewson: http://journeytoforever. org/biofuel_library/ethanol_manual/manual_ToC. html distillation