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Production of Bio-Ethanol from Biomass (Press Mud)

Sangram S. Pawar
Dept of Chemical Engineering, COE, Bharati Vidyapeeth University, Pune
sangrampawar19nov@gmail.com

Dr. Prakash Chavan
Dept of Chemical Engineering, COE, Bharati Vidyapeeth University, Pune
pvchavan@bvucoep.edu.in

Dr. Sandeep Bankar
Dept of Chemical Engineering, COE, Bharati Vidyapeeth University, Pune
sandip.bankar@aalto.fi

Abstract**: This document gives formatting instructions for authors preparing papers for publication in the Proceedings Bioethanol is renewable; eco-friendly energy source can be produced from biomass. Increasing the use of bio-ethanol for energy generation purposes is of particular interest now a day because they allow reducing the Greenhouse Gases (GHG). The production of low-cost ethanol by using lignocelluloses material basically the sugar industry wastes like bagasse and press mud. Press mud was characterized as moisture (70-75%), ash (18.25%) and protein content (1.68 g/L). Press mud contains sufficient amount of cellulose (22.3%) and hemicelluloses (21.67%) so it has an allure for use as a potential feedstock. Press mud drying study was also carried out to avoid microbial spoilage and increase storability. The acid hydrolysis of press mud with optimized parameters (H_2SO_4, 1.5 %; solid to liquid ratio 1:5 and time 15 min.) resulted in sugar release of 21 g/L. Furthermore, the activated carbon detoxification technique was carried out to remove phenolics, which are destructive to the fermentation process. The batch fermentation of detoxified press mud using Saccharomyces cerevisiae NRRL Y-12632 (pH-4.5, Temp- 28°C, RPM- 120, Time- 96 hrs.) resulted in 11.65 g/l of total solvents. Hence, clean and green, effective utilization of press mud for bio-ethanol production successfully achieved.

Keywords: Bioethanol, Fermentation, Press Mud, Pretreatment.

I. INTRODUCTION

With the developing of human lifestyle and civilization, emphasis on energy consumption increases day by day. For this reason different non-renewable energy sources such as coal, NG, fissile fuels etc. are the main target to fulfill human needs. This non-renewable energy sources are creating many environmental issues and this is the main cause of global warming. This all is the non-renewable energy sources are declared not remain much more in the reservoir to fulfill the growing demands of the world. For this reason, many of the countries of the world are trying to find out a suitable and sustainable solution to the growing demand for the fuel with the best consideration of the environment [21]. The resilience of lignocellulosic materials is due to their composition and physicochemical matrix. Lignocellulosic materials are composed principally of three components namely, cellulose, hemicelluloses, and lignin. There are liquid hot water, acid hydrolysis, enzymatic hydrolysis etc. methods are available for the pre-treating, hydrolysis and fermentation among these methods we have to select a method which giving more yield, safer and it is a less expensive [19].

The sugar industry waste i.e. press mud can be a good feedstock for biofuel production. India is the second largest producer of sugarcane in the world while India produces around 350-400 million tons annually and in season 2016-17 it seems near to 320 million tons [8]. In general, the sugar industry generates wastes such as a residue of cane left after cane harvesting, bagasse, press mud and spent wash. During sugar production, press mud is a Press mud is a soft, spongy, lightweight, amorphous, dark brown to the black colored .solid residue obtained before crystallization of sugar .3.6 to 4 % of press mud is left as a by-product after the crushing of 1 tons of sugarcane. India produces annually approximately 10-12 million tons of press mud as a waste and that can be utilized mostly as manure. It contains approximately 70 to 80 % water, 5-15 % sugars and other nutrients which make it a superior fertilizer [6]

Drying is a mass transfer process consisting of the removal of water or another solvent present in it by evaporation from a material. Press mud is being consolidated as one of the good abundant bio-resources from sugar industry in India for use as green energy.

Bioethanol derived from this waste. To minimize storage losses and to optimize the processing parameters during biochemical conversion. To get them, need to be dried due to their high moisture content.The drying operation is a simultaneous heat and mass transfer in which free water in the material is removed to a specific level at which microbial spoilage and deterioration chemical reactions are greatly reduced. The storage of these wastes until processing may be difficult. To overcome this problem, drying can constitute an efficient solution of preservation [3].So drying is vital in bioethanol production.

The purpose of pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity and increase the porosity of the material. The pretreatment must improve the formation of sugars, avoid the degradation of carbohydrates, avoid the formation of by-products inhibitory to the subsequent hydrolysis and fermentation process and be cost effective. Hence, pretreatment is an essential and fundamental step to disrupt lignin structure for optimal successful hydrolysis and downstream operations [22]. Physical, physicochemical, chemical and biological processes have been used for pretreatment for lignocellulosic material. Dilute acid hydrolysis as been successfully developed, using of sulphuric acid ($H2SO4$) becomes more advantageous than other pre-treatment's with optimized conditions, concentration of 1.5% (v/v), S:L ratio of 1:5 (g/ml) and pretreatment time of 15 min for higher sugar release[16]

The acid hydrolysis pretreatment process of lignocellulosic biomass generates various types of inhibitors such as furfural, 5-hydroxy methyl furfural (HMF) and phenolics. [15] These compounds are formed by decomposition of pentoses and hexoses while phenolics compounds are formed by degradation of lignin. It was demonstrated that *S. cerevisiae* is more sensitive to inhibition by furfural then HMF at the same concentration, while these inhibitors are suppressed the cell growth. [2] These compounds have negative effects on the downstream processes. Therefore, removal of these compounds is essential. To overcome the inhibitory effect on fermentation process, the hydrolysate needs to be detoxified. Several detoxification methods (physical, chemical and biological) have been used to converts inhibition compounds into inert materials or to reduce their concentration. The hydrolysate obtained from pretreatment is utilized as a substrate for microbial fermentation, where converting fermentable sugars to bioethanol by using *Saccharomyces cerevisaie* NRRL Y-12632. *S. cerevisaie* can grow aerobically on glucose, maltose, and trehalose and fail to grow on lactose and cellobiose [13]. This is very common in brewing beer when it is sometimes called a top-fermenting or top-cropping strain. *S. cerevisaie* has positive advantages in the industrial process due to its tolerance to alcohols and fermentation conditions. Whereas high production was achieved with bacterial systems, metabolic engineering of S. *cerevisaie.* Hence, it is successfully used in various industrial processes due to its high strength.

Thus the aim of this project work was to develop an economically viable process to produces ethanol from lignocelluloses material basically the sugar industry wastes press mud. To achieve Indian ethanol demand, it has potential to help achieve ethanol blending mandate of 20%. For green and clean fuel which significantly reduces greenhouse gases (GHG) emission.

II. MATERIAL AND METHODS

A. Materials

Sulphuric acid (H_2SO_4), Hydrochloric acid (HCl), Sodium chloride (NaCl), Sodium Hydroxide (NaOH), Potassium dihydrogen phosphate (KH_2PO_4), Ammonium sulfate ($(NH_4)_2 SO_4$) , Magnesium Sulfate ($MgSO_4$) 3,5- dinitro salicylic acid, ammonium acetate, sodium potassium tartrate, ethanol, sodium acetate and phenol, ammonium acetate, D-glucose, peptone, soluble starch, L-cysteine hydrochloride, these all chemicals are purchased from SRL Ltd, India, Avra Synthesis Ltd, India and Sigma-Aldrich, India. While Yeast extract was purchased from Himedia, India. All chemicals are analytical grade.

B. Microorganism and Its Maintenance

The strain of *Saccharomyces Cerevisaie* NRRL Y-12632 used in this work was freehearted gifted by Agriculture Research services, culture collection, USA. The *Saccharomyces Cerevisaie* maintained at 4°C on agar slants. The composition of agar slant was (g/l); yeast extract 3, malt extract 3, peptone 5, glucose 10 and agar 20. The culture was maintained by subculturing every 20-25 days.

C. Characterization of press mud

Fresh press mud sample was obtained from "Sahyadri Co-Operative Sugar Industry" Yashvantnagar, Karad, Maharashtra, India and it stored at 4-5°C until use. Proximate analysis of press mud was carried out after hot air oven drying at 80°C for 18 hrs. The moisture content and ash content was determined according to standard methods [20]. Accompanying, the lignin content was determined by Klason method [12]. Cellulose was estimated by a standard method and it quantified by Anthony reagent [16] while hemicellulose was estimated according to the standard method reported by Gao 2014.

D. Drying of Press mud

Drying of press mud was carried out at a temperature range between 60 to 100°C, time 10-18 hrs by using of hot air oven (Bio-Technic BIT-30, India) with a maximum output temperature of 200°C and the oven dimensions are 400mm-200mm-380mm (W-D-H). The drying was continued until all the moisture was removed (constant sample weight) from the press mud sample [9]. The moisture content and moisture ratio of press mud was calculated on web basis as,

1] $$\text{Moisture content} = M = \frac{(Wi - Wf)}{Wi}$$

Where Wi is the initial weight of press mud (g) and Wf is the final weight or press mud (after drying) (g)

2] Moisture ratio was calculated as, $MR = \frac{(M-Me)}{(Mo-Me)}$

Where M is a moisture content at any time t, Me is the equilibrium moisture content and Mo is initial moisture content of press mud.

Assume, Mo to be zero at drying process end. Therefore,

Moisture Ratio = MR = $\frac{M}{Mo}$

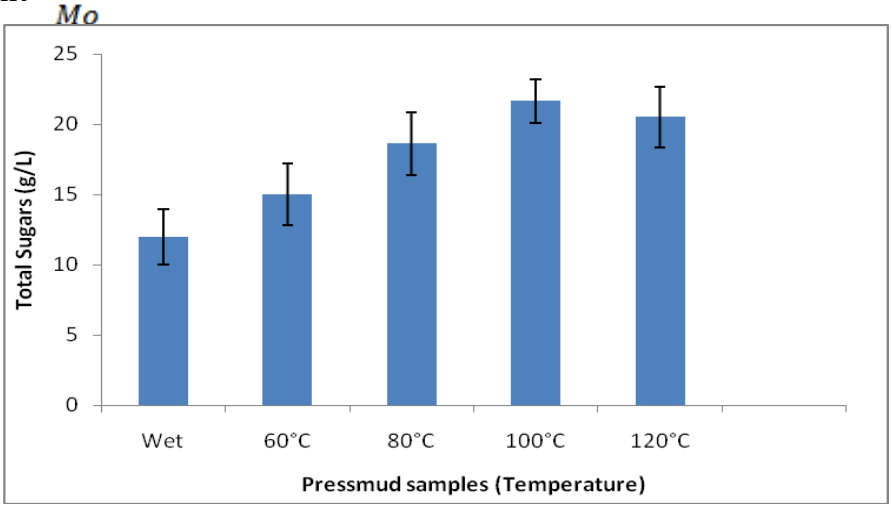

Fig 1 : Drying of Press Mud

E. Pretreatment - Acid Hydrolysis

Dilute acid hydrolysis was successfully developed for pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improves cellulose hydrolysis. The dried press mud was subjected to an acidic pretreatment for the breakdown of complex structure (cellulose, hemicellulose) of material. Acid pretreatment was carried out by optimized parameters as 20 gm of Press mud sample was mixed with 100ml of oil. Sulfuric acid (1.5% v/v) in a 250 ml conical flask with a solid to liquid ratio of 1:5. The conical flask was closed with cotton and silver foil and autoclaved at 121ºC for 20 min. After autoclave cooled it, the pretreated press mud slurry was taken out and filtered using filter paper. A neutralization of pH is necessary for further process or fermentation. The resulted filtrate (Hydrolysate) was used for subsequent sugar analysis and filtrate used for the further process.

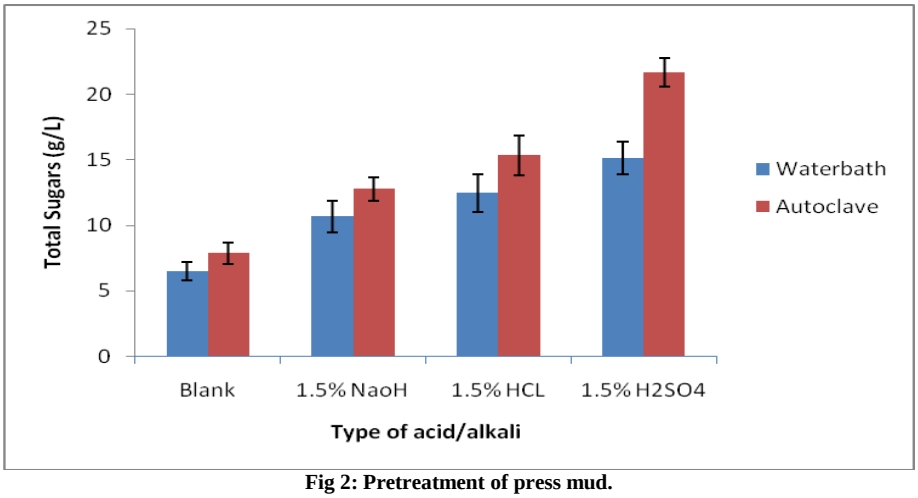

Fig 2: Pretreatment of press mud.

F. Detoxification – Activated Charcoal

Acid hydrolysis of lignocellulosic materials produces several inhibitory compounds, such as sugar and lignin degradation products, compounds derived from the lignocellulosic structure, and heavy metal ions. Their toxicity is a major factor limiting bioconversion processes that utilize hydrolysates. Detoxification of press mud hydrolysate was employed with activated charcoal with standard method [7].The pH of press mud hydrolysate (4.5) was gradually increased to 8 by using sodium hydroxide solution. After that, 5% (w/v) activated charcoal was added in the hydrolysate. The conical flask of a mixture of the hydrolysate and activated charcoal powder kept at continuous shell shaker at a temp of 60°C and 200 RPM for 2 hrs. The hydrolysate was separated by simple filtration and centrifugation. The filtrated hydrolysate again autoclaved for sterilization and was used for the fermentation process.

G. Fermentation Medium

The carbon source used was pressed mud hydrolysate with sugar concentration approximately 76.5 (g/l) with supplementation of glucose. In the batch fermentation, the medium was sterilized at 120°C for 20 min. and the pH was adjusted to 4.5.

H. Inoculum Preparation

The Inoculum was prepared cleanly in a two stages growth process. In the first stage, the *Saccharomyces Cerevisaie* was cultured in 250ml conical flask containing 100ml of growth medium(g/l); yeast extract 10, peptone 20, Dextrose 20 and pH is 6.5 incubating this on shall shaker at 28°C, 120 RPM for 12-16 hrs [18] Afterward the 5% medium was transferred to fermentation medium (g/l) ;glucose 76.5, yeast extract 4, Potassium dihydrogen phosphate (KH_2PO_4) 1, Ammonium sulfate ($(NH_4)_2 SO_4$) 1, Magnesium Sulfate ($MgSO_4$)5 and incubated in shall shaker at 28°C, 120 RPM for 24-96 hrs, pH 4.5 adjusted by using sulfuric acid. [23]

I. Ethanol Fermentation

Fermentation was carried out on press mud hydrolysate using *Saccharomyces Cerevisaie* NRRL Y-12632 for ethanol production. The standard fermentation medium was used expect press mud as a carbon source in present work. The press mud hydrolysate was supplemented with other nutritional components of fermentation medium and also synthetic sugar if necessary. The batch fermentation was carried out in 250 ml conical flasks with 100 0ml working volume and autoclaved at 121 ^oC for 20 min and cooled. The standard P medium was used as a control. After that flask are inoculated with actively growing culture (5% v/v Inoculum) and incubated for 96 hrs at 28 ^oC at 120 rpm. The samples were periodically taken out during fermentation with the help of sterile micropipette for ethanol analysis by using gas chromatography.

J. Analytical Methods

Ethanol sample was quantified as described by Bankar S.2013. Gas chromatography (Thermo Scientific Trace ^{TM} series 1110) equipped with a flame ionization detector and AB-INNOWAX capillary column (30 m × 0.32 mm × 1 µm) was used. The injector temperature was 200^oC and the detector temperature was 250^oC. The injector volume was 1 µL. By using double beam UV – spectrophotometer (UV-3000$^+$ from lab India), the reducing sugars were estimated by the dinitro salicylic acid (DNS) method (Miller G. et al 1959) using glucose as standard and the total sugars were quantified by Phenol-sulfuric acid method [4]

III. RESULTS AND DISCUSSION

A. Feedstock Characterization

To determine the composition of press mud so that it was characterized. Press mud contains cellulose 22.3%, hemicelluloses 21.67 % and lignin 12.90 % on dry basis. The moisture contains of press mud is 76.21% (w/w) ash content is 18.25 % (w/w) and protein content 1.65 g/l these values are correlated with other studied reported values of Gupta n.2011. In press mud, the percentage of cellulose and hemicellulose content shows, it has as a sufficient potential to be used as a feedstock for bioethanol production.

B. Drying of Press mud

The drying operation is important to avoid microbial spoilage and deterioration chemical reactions. The storage of press mud until processing may be difficult. To overcome this problem, drying can constitute an efficient solution of preservation of press mud. The press mud was dried at different temperatures (60°C, 80°C, 100°C, 120°C) using the hot air oven. Drying readings are taken after a time interval of 20min until total removal of moisture present in it. After drying it observed that, at a temperature of 100°C the concentration of total sugar is higher than other press mud dried at a different temperature.

C. Acid hydrolysis

Pretreatment is vital step to disrupt cellulose, hemicellulose and lignin matrix for successful hydrolysis and effective fermentation operation. On the basis of literature studied, the acid concentration, solid-liquid(S:L) ratio and time are important factors that affect total sugar yield in a pretreatment process of biomass [11]The acid hydrolysis was good and economical pretreatment as compare to other pretreatment methods. The acid (H_2SO_4) concentration of 1.5% (v/v), S:L ratio of 1:5 (g/ml) and pretreatment time of 15 min for higher sugar release. On the basis of drying kinetics of press mud, the dried press mud gives a total sugars then the wet press mud [16]. The total sugar release data obtained after acid hydrolysis process shows maximum total sugar released (21.24 g/l.) by using 1.5 % Sulfuric acid (H_2SO_4).

D. Detoxification of hydrolysates

The pretreatment and acid hydrolysis of lignocellulosic biomass process generate various types of inhibitors such as furfural, 5-hydroxy methyl furfural (HMF) and phenolics. The neutralization process which is used to maintain the initial pH of pretreated hydrolysate prior to fermentation also leads to precipitation formation that subsequently affects ethanol production. We detoxified hydrolysate by activated charcoal; it efficiently removed more than 90% phenolics with 8-10 % sugar loss. Total sugar of hydrolysate after detoxification was 19.95 g/l. The use of Activated Charcoal treatment for detoxification is cost effective and easy to do.

E. Fermentation

Dried press mud sample which was hydrolyzed and detoxified were used in the batch fermentation of *saccharomyces cerevisaie* NRRL Y-12632 to produce ethanol. Effective cell growth was obtained in fermentation medium by *saccharomyces cerevisaie* using the concentration of inoculums was 4.5. The fermentation experiment was performed minimum 96 hrs. The *S. cerevisaie* utilization of approximately 50-53% sugars during fermentation process proves the feasibility of press mud sample to be used for large-scale operation after certain modification. Ethanol a sample was analyzed by gaschromatogphy, resulted in ethanol concentration is 11.65(g/l)

CONCLUSION

Press mud was successfully used for the production of bioethanol by *saccharomyces cerevisaie* NRRL Y-12632. The total sugar yield and total solvents were higher for dried press mud than wet press mud. The press mud offered highest total sugar release of 21 g/l from sample dried at 100 °C. The activated charcoal detoxification efficiently removed more than 95% phenol and around 98% furan from all pretreated hydrolysate. By using *saccharomyces cerevisaie* NRRL Y-12632 fermentation of Detoxified hydrolysates resulted total ethanol concentration was 11.65 g/l. Hence we can utilize press mud sample to be used for large-scale operation after certain modification.

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