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Simultaneous Sachharification and Fermentation of Rice Residues and its Comparative Analysis for Bioethanol Production

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ABSTRACT

Energy consumption has inflated steadily over the last century because the world population has fully grown and additional countries became industrialised. Bioethanol is an alcohol produced by fermentation of plant biomass, containing carbohydrate and its production depends upon feedstock availability, variability, and sustainability. The selection of feedstock and its pretreatment is an important part of bioethanol production process. In present work, the exploration of the potential of agro-waste rice residues such as, rice bran and rice husk was done, because it contains sufficient amount of carbohydrate which can be ferment into bioethanol. The aim of the research was also to investigate how different pretreatment methods with moderate conditions differ in hydrolysis and fermentation efficiencies. Pretreatment plays an important role in the hydrolysis of cellulose and lignocellulose. It was found that biological pretreatment was a most effective method in terms of production of bioethanol and it enhances the production as well as fermentation efficiency.

Keywords: Bio-fuels; Feedstock; Fermentation; Rice bran; Rice husk.

1. INTRODUCTION

To meet the growing demand of energy for transportation and industrial processes, and to provide the raw material for industry in a sustainable way is one of the greatest challenges for society in the 21st century[1]. Due to the hike in the gas price and interest in environmental issues like global warming, increased demand for renewable energy. The quality constituents of ethanol match with fossil fuel so it can be used as blending with petrol and diesel. Bioethanol can be produced from any carbohydrate-containing substrates after fermentation. Biofuels can be classified on the basis of the substrate which is used as feedstock. First generation biofuels are made from feedstock contains sugar, starch, and cellulose. Since these feedstocks are a part of an animal or human food chain and they have undesirable competition for food supply. A switch to a more abundant inedible plant material should help to reduce pressure on the food crops[2].

Second Generation biofuels are produced from non-food crops such as wood, organic waste, food crop waste, and specific biomass crops, therefore eliminating the main drawbacks of first-generation biofuels. The third generation of biofuels is produced by algae which are a low-cost, high-energy and entirely renewable feedstock. Fourth Generation biofuels are aimed at not only producing sustainable energy but also a way of capturing and storing CO_2[3]. The selection of feedstock is an important part of bioethanol production. Some researchers used cereals like barley, maize, oat and sugar beet[4-9,29], jatropha oil cake, Azolla, waste fruits, deoiled rice bran and rice bran for bioethanol production.

As Chhattisgarh is known as the Bowl of rice due to supremacy in the production of rice. Rice residues serve as best candidates for bioethanol production due to their carbohydrate contents. Most rice varieties are composed of roughly 20 per cent rice husk, 11 per cent bran layers, and 69 per cent starchy endosperm, also referred to as the total milled rice. Each kg of milled rice produced results in roughly 0.7–1.4 kg of rice straw depending on varieties and moisture content during harvest. The rice husk, also called rice hull, is the coating on a seed or grain of rice. Rice residues like rice straw, rice husk, and rice bran are the abundant lignocellulosic waste materials which can be used as a substrate for the production of second generation bioethanol. Rice residues are lignocellulosic materials, having a sufficient amount of cellulose and hemicellulose. Due to being a polysaccharide pretreatment techniques are required to release sugars. The structure of lignin and cellulose is disrupted on pretreatment and sugar is released for ethanol fermentation. The present investigation deals with the use of agro-waste rice residues such as rice bran and husk for bioethanol production and comparative analysis of the various pretreatment techniques to enhance the production.

2. MATERIALS AND METHODS

2.1 Collection of Sample

The rice residues rice bran and husk were collected from the rice industry, Arihant Akshat Udyog, situated at village Labharakhurd, District- Mahasamund Chhattisgarh. Sample

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was air dried and kept in sterile polybags at room temperature for further use.

2.2 Microorganism

A strain of *Bacillus cereus McR*-3 was used for the fermentation of Rice husk to produce bioethanol.The strain of *Bacillus cereus McR*-3 was taken from School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur (C.G.) and it was maintained in Nutrient broth medium.

2.3 Fermentation Process

5 gram of Rice bran and husk sample was dipped 100 ml of distilled water and *Bacillus cereus McR-3* bacteria was inoculated and kept for incubation at 37 °C and 80 rpm, for 72 hours[9].

2.4 Estimation of Bioethanol

The ethanol was estimated qualitatively by Jones reagent ($K_2Cr_2O_7 + H_2SO_4$)[10], Formation of green colour indicates that the used carbon source produces ethanol on fermentation[11]. Qualitative estimation of ethanol was done by specific gravity method[12].

2.5 Optimisation of Pretreatment Methods

The aim of pretreatment is to increase the surface area and porosity, break the lignin components, removal of hemicelluloses and decrease the crystallinity of cellulose. In this study, rice bran and husk were subjected to acid and alkali pretreatment.

2.5.1 Acid Pretreatment

For this method dilute sulfuric acid was used for pretreatment. 5 g rice bran and husk were dipped into 95 ml of 1 per cent (v/v) sulphuric acid. It was autoclaved and the hydrolysate was filtered by whatman filter paper[13,30]. Residues were washed multiple times, by plenty of distilled water to make the pH neutral and it was kept for drying overnight at temperature 60 °C. The dried pretreated sample was dipped in distilled water and autoclaved. Inoculation of *Bacillus cereus McR-3* was done and fermentation was carried out for 72 hours. The amount of bioethanol production was estimated by specific gravity method. The same procedure was done with the subsequent 2 per cent, 3 per cent and 4 per cent of sulfuric acid.

2.5.2 Alkaline Pretreatment

For this pretreatment sodium hydroxide was used. This pretreatment is used to compare the better pretreatment technique for the enhancement of yield of more sugar for production of more ethanol. It was seen that liberation of sugar was more in alkaline pretreatment in comparison with acid pretreatment.

The hydrolysate was prepared with NaOH of 1 per cent residues were washed to make the pH neutral and it was kept for drying overnight at temperature 60 °C. The dried sample was dipped in distilled water and autoclaved[14]. Inoculation of *Bacillus cereus McR-3* was done and fermentation was carried out. The amount of bioethanol production was estimated by specific gravity method. The same procedure was done with subsequent 2 per cent, 3 per cent and 4 per cent sodium hydroxide.

2.5.3 Biological Pretreatment

In biological pretreatment, lignocellulosic substrate is treated with enzymes or whole organism. In the present study, *Aspergillus niger* was used for pretreatment, because it releases, exoenzyme cellulase which hydrolyzses the cellulose of rice bran and husk into fermentable sugar. Bacterial culture of *Bacillus cereus McR 3* was inoculated in each flask and fully fledged mat of *Aspergillus niger* was also inoculated into it. It was kept for fermentation at 37 °C and 80 rpm for 7 day. Total sugar and reducing sugar was estimated by Anthrone and DNS methods respectively[15].

2.5.4 Ethanol Yield, Ethanol Productivity and Fermentation Efficiency

Fermentation efficiency is an expression of how much ethanol was actually produced relative to the amount that could be theoretically produced. Ethanol yield is a parameter that is calculated as the amount of ethanol produced per unit of substrate utilisation. Ethanol productivity is estimated as the amount of ethanol produced per unit of substrate utilised in one unit of time[16].

2.5.5 Statistical Analysis

The data of bioethanol produced were statistically analysed using ANOVA (one –way analysis of variance) by SPSS (Statistical Package for Social Sciences) version 16.0. The significant difference in ANOVA ($p = 0.05$) was assessed by Duncan's Multiple Range Test and all the experiments were performed in triplicates. Means and standard error (±SE) of the readings were taken.

3. RESULTS AND DISCUSSIONS

The main objective of the work was to study bioethanol production from rice bran and rice husk using *Bacillus cereus McR*- 3 and optimisation of various pretreatment methods to enhance the bioethanol production.

3.1 Fermentation of Rice Residues and Calculation of Fermentation Efficiency, Ethanol Yield And Productivity

Rice residues, rice bran and husk were fermented with the inoculation of *Bacillus cereus McR*-3. The total sugar estimation was performed by anthrone method and dinitrosalycylate method. It was seen that the amount of total sugar was more before fermentation, the microorganism utilises more sugar during the fermentation process also produced a high amount of ethanol. Arumugam and Manikandan[17] worked on banana and mango fruit waste for ethanol production and found 64.27 per cent of maximum reducing sugar production in mixed fruit pulps, after dilute H_2SO_4 pretreatment. Abo-state[18], et al. have found maximum reducing sugars of 12.62, 13.58, 17.00 g/L by pretreatment with fungal isolates F68, F94 and F98 respectively. Deswal[19], et al. obtained 157.160 mg/g and 214.044 mg/g of reducing sugar from Fomitopsis sp. RCK 2010 pretreated rice

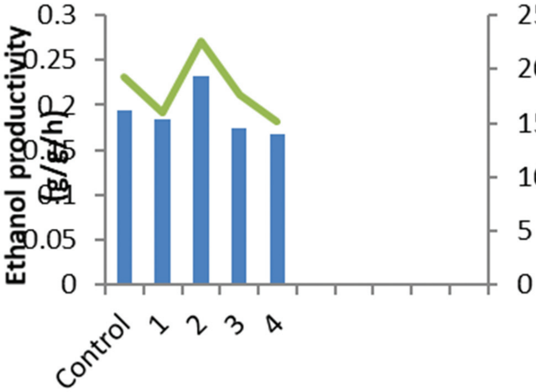

Figure 1. Ethanol yield and productivity of rice bran with acid pretreatment.

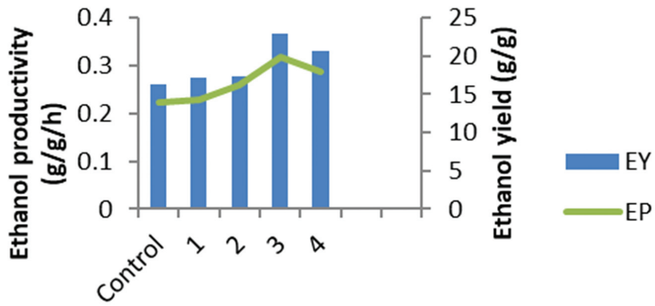

Figure 2. Ethanol yield and productivity of rice bran with alkaline pretreatment.

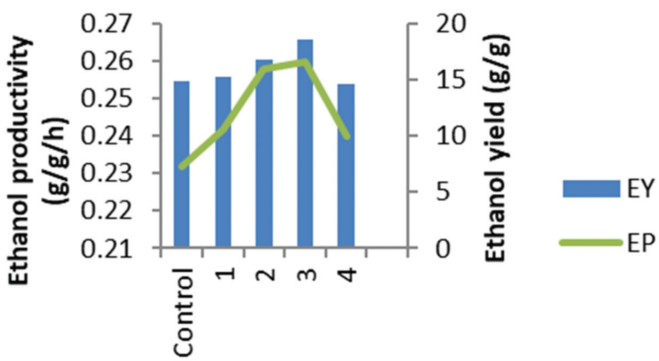

Figure 3. Ethanol yield and productivity of rice husk with acid pretreatment.

husk and wheat husk respectively. Upendra[20] have obtained 90 g of glucose/kg of agro- waste. Talebnia[21], et al. achieved 74-99.6 per cent of sugar yield after enzymatic hydrolysis of wheat straw. Wei[22], et al. achieved the maximum yield of sugars from 1g of rice hulls by acidic treatment under optimised condition was 213.6 mg, the amount of sugars obtained from 1 g of pretreated rice hulls by enzymatic saccharification was 307.7 mg. The fermentation efficiency was 82.44 per cent in 2 per cent acid pretreatment for rice bran. In the case of rice husk after pretreatment with 3 per cent H_2SO_4, the amount of sugar consumed and fermentation efficiency was 91.20 per cent in comparison to other pretreatment methods. It was observed that consumed sugar was highest in 3 per cent NaOH pretreatment

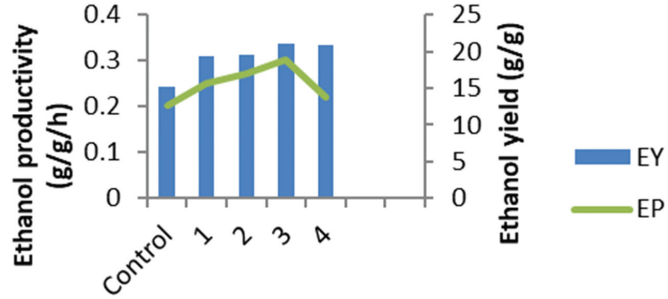

Figure 4. Ethanol yield and productivity of rice husk with alkaline pretreatment.

for both rice bran and rice husk and fermentation efficiency was 91.84 per cent and 95.70 per cent for rice bran and rice husk respectively. The consumption of sugar was highest in biological pretreatment in both cases for rice bran and husk. Ethanol yield and productivity was also estimated and in case of rice bran ethanol yield and productivity was highest 0.19 g/g and 0.274 g/g/h respectively at 2 per cent acid pretreatment. The value of ethanol yield was 0.22 g/g and productivity was 0.317 g/g/h for rice bran with the alkaline pretreatment of 3 per cent. Ethanol yield by rice husk was 0.18 g/g and productivity was 0.258 g/g/h for acid pretreatment at 3 per cent. The value of ethanol yield and productivity was highest at 3 per cent alkaline pretreatment, 0.21 g/g and 0.301 g/g/h. The ethanol yield and productivity was most at biological pretreatment, it was 0.24 g/g and 0.337 g/g/h for rice bran and 0.23g/g and 0.322 g/g/h for rice husk (Figs. 1-4).

3.2 Acid Pretreatment and Amount of Bioethanol

The pretreatment changes the native properties of the substrate in order to prepare the materials for enzymatic degradation. Among all acid pretreatment 3 per cent was given maximum production of bioethanol from rice husk 9.30 ± 0.09 and 2 per cent acid pretreatment gave highest production of bioethanol, 9.89±0.09 per cent for rice bran (Table 1). Srivastava[14], et al. (2014) have worked on dilute acid pretreatment and obtained the maximum ethanol was 3.20 ± 0.36 g/l.

3.3 Alkaline Pretreatment and Amount of Bioethanol

Alkaline pretreatment also enhances the production of ethanol. In both cases, 3 per cent NaOH pretreatment gave maximum production of bioethanol, 11.43±0.08 per cent for rice bran and 10.85± 0.09 per cent for rice husk. The value of 3 per cent treatment was significantly different from 1 per cent, 2 per cent, and 4 per cent treatments (Table 1). Nikzad[13], et al., worked on pretreatment on rice husk and they have found maximum ethanol concentration for dilute- NaOH was 6.22g/l. Saha and Cotta [23] obtained, 8.2 ± 0.2g/l concentration of ethanol from fermentation of Alkaline H_2O_2 pretreated rice husk by *E.coli*. Saha and Cotta [24] obtained 9.8 ± 0.5 g/l amount of ethanol from lime pretreated rice husk on fermentation with ethanol. Diaz[25], et al. obtained the highest yield of 86.48 ± 3.07 per cent after pretreatment of alkaline peroxide on rice husk.

Table 1. Effect of acidic and alkaline pretreatments on bioethanol production by rice bran and husk

Pre treatment	Bioethanol (%) Rice bran	Bioethanol (%) Rice husk
Control	8.92±0.12	7.67±0.18
1% H_2SO_4	8.12±0.08[b]	8.40±0.23[b]
2% H_2SO_4	9.89±0.09[c]	9.21±0.09[c]
3% H_2SO_4	7.49±0.01[a]	9.30±0.09[c]
4% H_2SO_4	7.36±0.06[a]	7.29±0.19[a]
1% NaOH	10.05±0.09[b]	9.03±0.09[a]
2% NaOH	10.20±0.42[b]	9.86±0.09[b]
3% NaOH	11.43±0.08[d]	10.85±0.09[c]
4% NaOH	10.80±0.01[d]	10.23±0.28[b]

3.4 Biological Pretreatment

Biological pretreatment is an effective, cheap and environment- friendly method. Biologically pretreated sample gives 12.16 per cent of bioethanol production from rice bran and 11.61 per cent of bioethanol from rice husk. It was observed that biological pretreatment is more effective than chemical pretreatment and this pretreatment supported the fermentation process the most. Pandey[15], *et al.* have worked on biological pretreatment of substrate Azolla for ethanol production and obtained the highest value of bioethanol 4.06 per cent (w/v). Afifi[26], *et al.* have also performed the biological pretreatment with *Aspergillus niger* and fermented with *Saccharomyces cerevisiae* reported the percentage of bioethanol as 2.33 per cent, 3.50 per cent, 4.80 per cent and 5.50 per cent after 18 h, 36 h, 72 h and 96 h incubation respectively. Ahmad[27] (2014) worked on bioethanol production from cellulose in red algae *Gracilaria verrucosa* by using pretreatment of *Trichoderma viride* and *Zymomonas mobilis* and found 1 kg of *Gracilaria verrucosa* produces 23.01 per cent bioethanol with the concentration of 29.60 per cent. Patel[28], *et al.* have worked on pretreatment of wheat straw and rice straw with *Aspergillus niger* and *Aspergillus awamori* and fermentation yielded highest amount of ethanol for rice husk.

4. CONCLUSIONS

In the era of globalisation, the increasing demand for energy and the dependence of countries on energy indicate, the search for new alternative and renewable source of energy. Lignocellulose has been identified as the prime source of biofuels and other value-added products. In the present study the potential of rice residues such as rice bran and rice husk has been explored for production of bioethanol, as it is easily available, cheap and contains the high amount of carbohydrates which can be fermented. It was found that biological pretreatment is most suitable for conversion of lignocellulose to simple sugar and rice bran gives more production in comparison to rice husk. These results may be helpful in optimisation of pretreatment techniques and bioethanol production process.

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Contributed in overall experiment design.