Biofuel Production Using Ionic Liquids

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Abstract: Lignocellulosic materials are abundant feedstock for biofuel production. The complex structure of the lignocelluloses is the main obstacle in the conversion of lignocellulosic biomass into valuable products. A promising new pretreatment method for lignocellulosic materials is the use of Ionic liquids (ILs). Ionic liquids provide the opportunities for their efficient pretreatment for biomass. Therefore, in this work, biomass pretreatment with ionic liquids (1-butyl-3- methylimidazolium chloride [BMIM]. Fermentation of banana waste to ethanol by saccharomyces cerevisiae was investigated at different temperature, different pH, and different incubation period and at different agitation speed.

Key words: Optimization; Ethanol, Saccharomyces cerevisiae, Ionic liquids, Fermentation.

Introduction

As the population increases, the need for an integrated system of different forms of renewable energy has increased earnestly. The limitation of fossil fuels and the global climate change are becoming major concerns due to the extensive use. Moreover, the enormous consumption of fossil fuels in the daily life has resulted in gas emission and led to several environmental problems[1]. The only current sustainable source of organic carbon is plant biomass. Biomass is defined as organic matter available on a renewable basis. Biomass includes forest and mill residues, agricultural crops and wastes, wood and wood wastes, animal wastes, livestock operation residues, aquatic plants, fast growing trees, and municipal and industrial wastes[2].

During the process of lignocelluloses-to-biofuel, the complex structure of the lignocelluloses is the main obstacle in lignocellulosic conversion. Different methods have been adopted to treat lignocellulosic biomass prior to use for bioethanol fermentation. Those methods include physical, chemical, biological and physico-chemical treatments. Such as grinding and milling, hot water, alkali and acidic treatment and biological treatment. However, there are several drawbacks associated with these techniques such as severe reaction conditions, high energy consumption, toxicity, expensive operation disposal recovery cost and slow reaction rate. Pretreatment of lignocelluloses in high temperature and high pressure is always required in order to break the lignocellulosic structure to make its cellulose available for enzymatic hydrolysis. It is necessary to develop alternative new techniques. In recent years, ionic liquids (ILs) have gained wide popularity for their increasing applications as they possess a number of interesting properties such as low vapor pressure, high thermal stability, and lack of flammability[3,5].

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The actual work deals with the process bioconversion of cellulose from Banana Pseudo stem waste, obtained from an agricultural waste, into ethanol by using the methods of acid pretreatment, hydrolysis and fermentation by Saccharomyces cerevisiae.

Materials and methods

Sample preparation:

The gathered banana stem were chopped into small pieces approximately 2-4 cm in length using knife. The pieces were then sun dried under mild sunlight for two days and then dried at 60°C in an oven for one day. The cut pieces were then crushed in the grinder. Saccharomyces cerevisiae (wild strain) and Saccharomyces cerevisiae NCIM 3495 are used in this study. Wild strain was obtained in commercial stores and Saccharomyces cerevisiae NCIM 3495 was collected from National Collection of Industrial Microorganisms (Pune, India)[6,7].

Steam pre-treatment:

The separate samples autoclaved at 15psi pressure for 30 min. After autoclaving the sample allowed to cooled then filtered.

Biomass pretreatment with IL:

1ml of IL and 250mg of biomass were mixed and heated at 120°C for 6h under continuous stirring.

Acid hydrolysis:

Diluted sulfuric acid was added to the sample from pretreatment steps. The sample was hydrolyzed in the reactor between 100 °C for 30 min. After hydrolysis, pH adjustment was carried out with 1M NaOH until the pH reached a pH of 7. Insoluble particles were separated from the hydrolysate by filtration[8].

Fermentation:

The 5g of yeast culture was added into the flasks. The samples were placed in shaker incubator at 200 rpm at 30°C, for 3 days.

Distillation:

Distillation is the most dominant and recognized industrial purification technique of ethanol. The basic principle that by heating a mixture, low boiling point components are concentrated in the vapor phase. By condensing this vapor, more concentrated less volatile compounds is obtained in liquid phase. Water is obtained from the bottom of the tower and ethanol is obtained from the top of the tower.

Growth curve:

Saccharomyces cerevisiae (wild strain) and Saccharomyces cerevisiae NCIM 3495 were inoculated in nutrient broths and they were consider as pre-inoculums and incubated for overnight. 1ml of sample from pre inoculated culture was added to fresh nutrient broth and they were considering as inoculums. In inoculums the growth of microbes were monitor for 4days at 2hours time interval using UV Spectroscopy at 600nm.

Analytical methods:

Ethanol concentration was determined by the Standard potassium Dichromate method[9,10].

Results and Discussion

Growth Curve for Sacchromyces cerevisiae (baker yeast):

The samples were collected at the interval of 2hours for 4days and the concentration of cells was calculated by absorbance at 600nm.
Growth Curve for Saccharomyces cerevisiae NCIM 3495:

The samples were collected at the interval of 2 hours for 4 days and the concentration of cells was calculated by absorbance at 600 nm.

Estimation of ethanol by steam pre-treatment:
Estimation of ethanol by ionic liquids:

Effect of temperature:

The results in Fig 5 indicate that maximum ethanol yield of 0.762 mg/ml was produced at temperature of 30°C. The ethanol yield increased with the increase of temperature from 20-30°C up to 48 h of incubation after which is declined. Thus, optimum temperature of fermentation of banana stem was found to be 30°C with the maximum ethanol yield 0.762 mg/ml at 48 hr incubation. Therefore, the future experiments were conducted at incubation temperature of 30°C.

Effect of pH:

Fig 6 shows the effect of pH on ethanol production. The ethanol yield increased with the increase of temperature from 4-6 up to 48 h of incubation after which is declined. Thus, optimum pH of fermentation of banana stem was found to be 6 with the maximum ethanol yield 0.802 mg/ml at 48 hr incubation [13.14].
Effect of incubation period:

In this study carried out on effect of incubation period on ethanol productivity. Saccharomyces cerevisiae exhibited maximum ethanol yield (0.638mg/ml) at 5 days of incubation[15].

Effect of agitation speed:

Fig 7 shows that effect of agitation speed on ethanol production. It is clear from the fig that lower agitation speed was found to be suitable for ethanol production. The highest ethanol yield of 0.74mg/ml observed with 200rpm of agitation which is primarily due to initial oxygen requirements of yeast cells. Excess oxygen in the fermentation medium could lead to increased cell growth at the cost of ethanol productivity[11,12].
Conclusion:

This study could establish that banana stem which have not been exploited commercially for any industrial application and are poorly disposed could effectively be used for ethanol production through the process of ionic liquids pre-treatment and fermentation. Ionic liquids treatment can significantly reduce the volume of the waste material. The process with optimized fermentation parameters described in the project could be used for scaling up of the process to a pilot scale or commercial fermented level thereby making the process more cost effective.

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