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Determination of optimum period of Saccharification of water hyacinth using Trichoderma Reesei and Aspergilus Niger

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Abstract

Alternative energy sources like bioethanol have become increasingly important in the recent past due to rapidly depleting oil reserves. Lignocellulosicfeedstocks are considered as an attractive raw material for bioethanol synthesis. Water hyacinth, an aquatic weed, tolerates variation in pH and temperature and is unaffected by toxic substances. It has high growth rates and creates problems to ecosystems and irrigation, which have called for control measures. It is found to be a suitable substrate for bioethanol production due to high cellulose and low lignin content. The two major steps involved in the conversion processes are Saccharification and Fermentation. This paper focuses on the experimental work carried out in the Saccharification stage. Pretreated Water hyacinth was added to the growth media and inoculated with Saccharification micro-organisms Trichoderma Reesei and Aspergillus Niger in different conical flasks. The optimum Saccharification period for both micro-organisms was found to be 72 hours. On testing, it was found that both organisms were capable of producing glucose, with Trichoderma Reesei (275 µg/ml) giving a better yield than Aspergillus Niger (175 µg/ml).

Keywords: Saccharification, water hyacinth, ethanol, pre-treatment, glucose.

Introduction

The global oil supply is practically constant because the natural formation of petroleum is a process which takes a very long time and it isn't replaced at the rate at which it is being extracted. The use of conventional fuels as primary energy source has led to scarcity in fuel, climate change, environmental degradation and human health problems[1]. These increasingly evident problems have resulted in an impending global energy crisis. As a result bioethanol is being considered as a potential liquid fuel to counter the limited natural resources. Cellulose biomass is also being investigated as a potential substrate for bioethanol production[2]. Lignocellulose is considered as a feasible raw material for the production of fuel ethanol, because of its availability in large quantities at low cost[3-4]. Currently, lignocellulosic materials can be obtained from bio-energy crops or bio-wastes.

The attributes of a bio-energy crop are as follows[5]: i. Naturally grown vegetation, grows perennially. ii. High cellulose and low lignin content per unit volume of dry matter. iii. Easily digestible.

Water Hyacinth (WH) is a widely prevalent aquatic weed in India. It constitutes a potential biomass resource for various uses. Its high content of hemicellulose (30–55% of dry weight) can provide hemicellulosic sugars for bio- conversion to ethanol[6]. The conversion of WH to ethanol is achieved in three major steps, namely, Pretreatment of the raw material, Saccharification of complex sugar molecules to simple sugars and finally Fermentation of the produced simple sugars into bioethanol[7]. A typical process description[8] is given by the flow chart in figure 1.

Pretreatment is the process by which cellulosic biomass is made amenable to the action of enzymes. All naturally occurring cellulosic materials require pretreatment to become accessible to the enzymes that control hydrolysis[9]. Saccharification yields in the absence of pretreatment are usually lesser than 20% of theoretical yields, whereas yields after pretreatment often exceed 90% of theoretical. Some of the common methods of pretreatment are acidic pretreatment and alkaline pretreatment. Pretreated Water Hyacinth (PWH) thus obtained is taken as the substrate. The second step is the hydrolysis of polysaccharides to soluble sugars, which is also called "Saccharification". In this step, the complex sugars are broken down to a simpler form, usually by the action of enzymes (cellulases), produced by the Saccharification micro-organisms such as Trichoderma Reesei and Aspergillus Niger. Fermentation is a process in which enzymes produced by microorganisms catalyze chemical reactions that break simple sugars into lower molecular weight materials such as organic acids and neutral solvents such as ethanol. Alarge variety of bacteria, yeasts, and fungi are of interest for fermentation[10].

Material and Methods

Sample collection: Water hyacinth used for the experiment was collected from the nearby Kengeri Lake (Bangalore, Karnataka). The leaves were separated from the stalks and kept aside for pre-treatment.

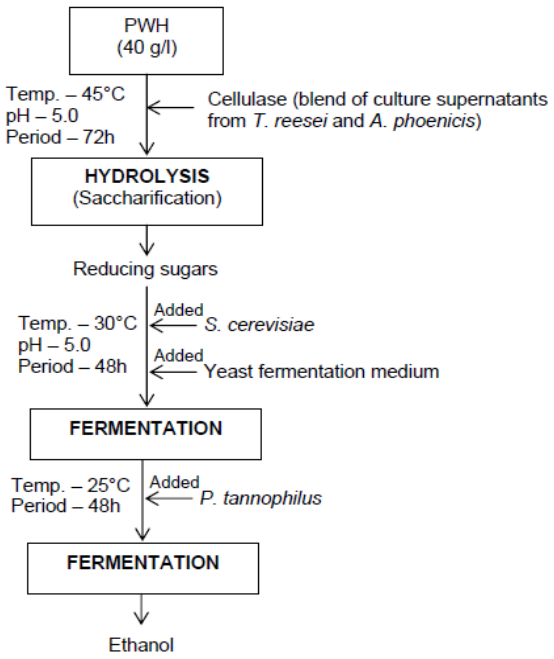

Figure-1
Flow Chart for conversion of water hyacinth to bioethanol[9]

Sample pre-treatment: The Water Hyacinth leaves were chopped into smaller pieces. The pieces were soaked in 0.1 N Sodium Hydroxide solution for 48 hours. The soaked pieces were then dried in the sun for 7 days. The sun dried pieces were then dried in a hot air oven for 7 hours to eliminate the moisture content and reduce it to a minimum level. The substrate was then subjected to mechanical communition. The resulting pieces were sieved in a 22 mesh and the underflow was taken as the substrate. The final substrate is shown in figure 2.

Preparation of media and flasks: The media was prepared by dissolving the Magnesium Sulphate (M_gSO_4) 1.5 g, Potassium DiHydrogen Phosphate (KH_2PO_4) 3g, Ammonium Sulphate ($(NH_4)_2SO_4$) 60 g. and (*NaCl)* 1.5 g. in 3litres of distilled water. 18 standard 150 ml conical flasks were taken and two sets were made, each set containing 9 flasks. Each set was further divided into 3 sets, with flasks being named 1, 2 and 3. 50 ml of media was added to each conical flask. 0.5g. of pretreated water hyacinth was added to each of 9 flasks (set 1) containing the media and 0.5g of Carboxy methyl cellulose was added to each of the other 9 flasks (set 2). The flasks were plugged firmly with cotton, sealed and autoclaved at 25 psi for one hour.

Inoculation of micro-organisms: The autoclaved media was brought to room temperature by allowing it to cool for 2 hours. The flasks numbered 1 from each set were inoculated with Trichoderma Reesei (WH-1 and CMC-1). The flasks numbered 2 from each set were not inoculated with any micro-organism and were taken as Control (WH-2 and CMC-2). The flasks numbered 3 from each set were inoculated with Aspergillus Niger (WH-3 and CMC-3). These flasks plugged with cotton to provide anaerobic conditions and were agitated on an orbital shaker at 100 rpm at 30°C.

Quantitative analysis of glucose: The Dinitrosalicylic Acid test was conducted at intervals of 24 hours. 1.5 ml of the contents of each flask was transferred to correspondingly labelled centrifuge tubes using a micro pipette. The centrifuge tubes were centrifuged at 1200 rpm for 20 minutes. The contents of the centrifuge tubes were allowed to rest for half an hour.

DNS Test: Using a micro pipette, 2 ml of the supernatant liquid was taken and transferred into a test tube. To this, 2 ml of Dinitro Salicylic acid reagent (DNS Reagent) was added and the contents of the test tubes are heated in a water bath. The heating was continued till the contents turned reddish in color. The procedure was repeated for the remaining 5 flasks. The control solution was taken and placed in the spectrophotometer/colorimeter. It was calibrated to zero. The Optical Density (OD) values of the cellulose flasks were obtained. The procedure was repeated for the Water hyacinth flasks also. The optical density values were noted down. The test was carried out at intervals of 24 hours. The media flasks were kept in the orbital shaker for the entire duration of the project.

Results and Discussion

The Optical Density (OD) is proportional to the concentration of the colored component in the test solution. This relation is given by the Beer-Lamberts law. The determination of the concentration of glucose was done by the DNS test where the OD of the sample was determined using a colorimeter. DNS test was carried out at time intervals of 24 hours. A glucose standard curve was prepared by taking known concentrations of glucose solution and these were plotted against the corresponding OD values. OD was measured at 540 nm since the absorbance is found to be highest at this wavelength. This standard curve was then used calculate the quantity of glucose produced in the experiment over 3 days. The day of inoculation was taken as the 0th day. The calculation was done by measuring the OD values obtained for each sample and calculating the corresponding glucose content. Table 1 gives the concentration of glucose used in different solutions to calibrate the glucose standard curve. The glucose standard graph thus obtained by plotting the standard optical density (OD) values is shown in figure 3.

Figure 3 was used to calculate the concentration of glucose produced on different days by measuring the OD of the glucose sample and finding the corresponding concentration values. The results were as follows tabulated in table 2.

Conclusion

During the course of the Saccharification process, increase in biomass was observed in each flask except those of control. Trichoderma Reesei was the higher yielding culture with a peak

glucose production of 275 µg/ml observed on day 2. Aspergillus Niger was the lower yielding species with a maximum glucose production of 175 µg/ml on day 2. The OD values increased for the first two days and dropped on the third day. Hence it was found that the optimum time of saccharification of the pretreated water hyacinth using T. Reesei and Aspergillus Niger was 2 days.

Figure-2
Pretreated Water hyacinth powder

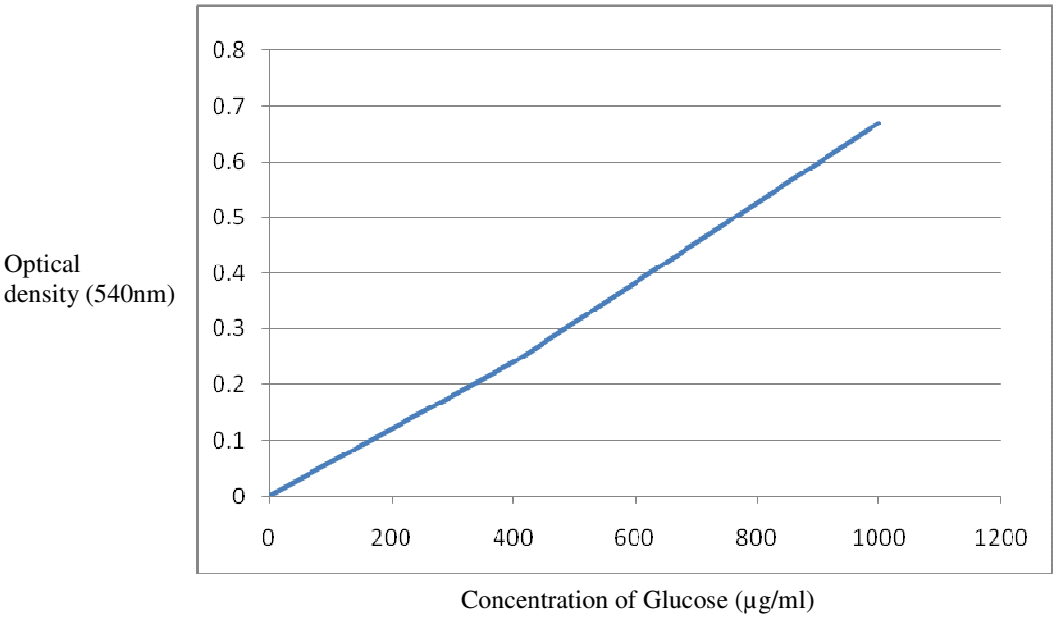

Figure-3
Standard glucose curve by DNS method

Future Prospects: The experimental study suggests Trichoderma Reesei and Aspergillus Niger can be used to synthesize glucose from Water hyacinth. The optimum saccharification period is 2 days from inoculation. The results from this phase are to be incorporated in the second phase of bioethanol synthesis, namely, the Fermentation of the obtained glucose to ethanol. Other prospects include variation of temperature to obtain optimum saccharification temperature for the given cultures. The experiment can be carried out using genetically modified strains of micro-organisms in an effort to obtain better yield.

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