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Effect Of Inducers In Ethanol Production Using Recombinant Bacterial Strain Zymomonas mobilis CP4

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Abstract: Bioethanol is a fuel used as a petrol substitute for road transport vehicles. Water Hyacinth (WH) which is a cheap lignocellulosic material (Cellulose, Hemicellulose and Lignin) found in ponds is used as a substrate in ethanol production. WH is pretreated using Acid hydrolysis (10%) followed by Detoxification. Ethanol is obtained from Water Hyacinth by Simultaneous Saccharification and fermentation (SSF) where saccharification is done by using cellulase enzyme and fermented using *Zymomonas mobilis CP4* (recombinant Strain) which can ferment both glucose (from cellulose) and xylose (from hemicellulose). The benefit of using SSF is that it is a speedy process with a minimum consumption of time and cost. Effect of inducers $FeSO_4.7H_2O$, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$ and $MgS0_4.7H_2O$ of ethanol production was studied using Plackett-Burman design. It is observed that ethanol is increased from 64.47g/l to 82.1g/l when supplemented with these salts.

Keywords: Water Hyacinth, Zymomonas mobilis CP4, ethanol, simultaneous saccharification and fermentation (SSF), Plackett-Burman design.

Introduction

Ethanol contains 35% oxygen and on combustion reduces particulate and NOx emissions. Hence it can be employed in the place of petrol[(1)]. Water hyacinth (*Eichhornia crassipes*) is a fresh water aquatic plant which is a new crop for bioethanol production. WH is a cheap lignocellulosic material that consists of Cellulose, Hemicellulose and Lignin[(2)] .*Zymomonas mobilis CP4* is a recombinant strain which can ferment both glucose and xylose. This contains the *E.coli* genes for xylose assimilation (xylose isomerase and xylulokinase) and pentose metabolism (transketolase and transaldolase) on the plasmid pZB5[(3)].The traditional method of optimization of parameters involves optimizing one parameter at a time, this is time consuming and involves several experiments to determine the optimum levels, which may not give exact values. To overcome these drawbacks, response surface methodologies of experimental designs like Plackett Burman method is used. This design allows testing of the effect of inducers with least number of observations. This design used to determine the metal ions that contribute significantly for ethanol production.[(4)]

Experimental

Materials and methods

Water Hyacinth used is pretreated by 10% Acid Hydrolysis autoclaved at 121^0C, 15lbs for 15minutes and Detoxified using $Ca(OH)_2$ and

NaOH. Xylose and Glucose assay was done before and after acid treatment[5]. The supernatant was collected and then used for fermentation process.

The production media for the *Z.mobilis CP4* is Yeast extract - 10g ; Agar - 15g; Distilled water- 0.9L; Autoclave at 121^{0}C for 15minutes and add sterilized 100ml of 20 %(w/v) glucose solution and 10ml of each stock solutions. Stock solution: Glucose -20g/100ml; $MgCl_2$ -10g/100ml; KH_2PO_4 -10g/100ml; $(NH_4)_2SO_4$-10g/100ml.

Simultaneous saccharification and fermentation was done. In saccharification process 5ml of cellulase enzyme was added which breaks cellulose into glucose units. Fermentation was done using *Zymomonas mobilis CP4*. In the production media the Carbon source was replaced by Water Hyacinth and SSF process was carried out. The ethanol produced is estimated by Distillation process and Dichromate assay[7]. By adding the inducers of different concentration to the fermentation media there was an increase in the yield of ethanol. This was done by Plackett Burman method. 9 runs were carried out with four metals of different concentrations [4]

Results and Discussion

Composition of water hyacinth estimated was found to be Hemicellulose- 42% Cellulose- 30% and Lignin- 11% [6]. Pretreatment of Water Hyacinth was done with 10% Acid Hydrolysis where Cellulose breaks to give glucose and Hemicellulose breaks to give Xylose. Acid hydrolysis is followed by detoxification, where the toxic substances formed during pretreatment are removed [5]. Instead of Batch fermentation SSF is done, which is a speedy process with reduced cost. In SSF process 5 ml of cellulase enzyme was added to break cellulose into glucose for better yield, and the carbon source of the production media for *Zymomonas mobilis CP4* was replaced by Water Hyacinth. Figure 1 shows ethanol was produced gave a maximum concentration of 64.4 g/l in about 34 hours. Ethanol produced was estimated using Potassium dichromate assay.

Inducers are added to enhance the productivity of ethanol. The inducers chosen for this study, with their concentration range as follows (g/l): $FeSO_4.7H_2O$ (0.002 to 0.006), $MnCl_2.4H_2O$ (0.002 to 0.006), $ZnSO_4.7H_2O$ (0.002 to 0.006) and $MgSO_4.7H_2O$ (0.001 to 0.005).

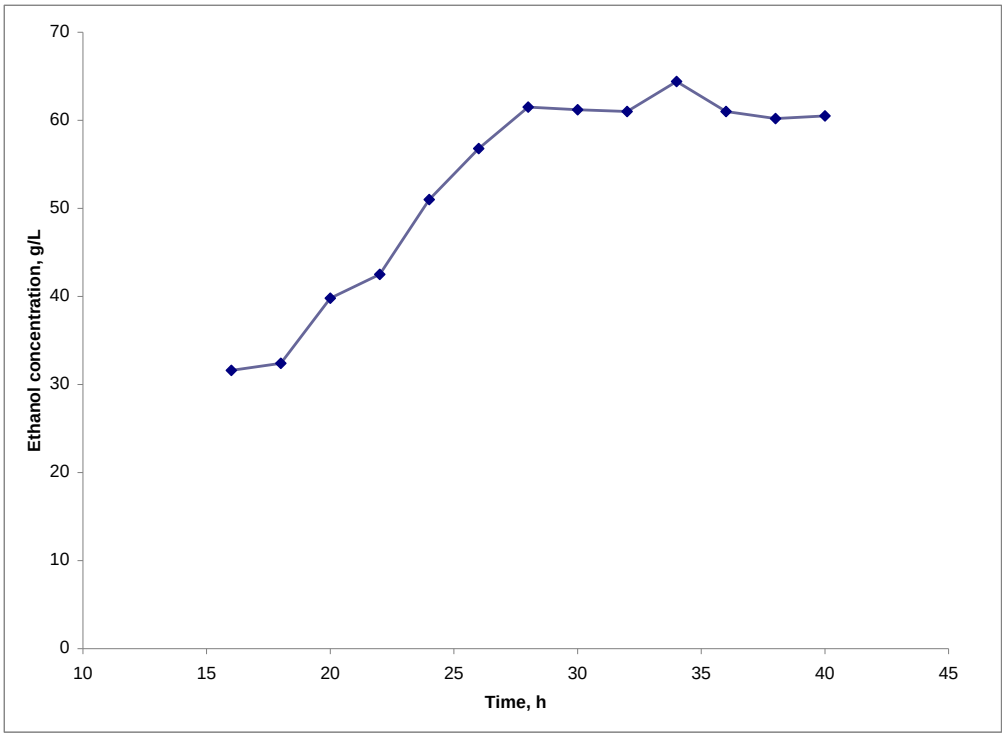

Figure 1. Ethanol production from SSF

Table 1.: Plackett and Burman fractional factorial design

Run	$FeSO_4.7H_2O$	$MnCl_2.4H_2O$	$ZnSO_4.7H_2O$	$MgSO_4.7H_2O$	Ethanol concentration(g/l)
1	0.002	0.002	0.006	0.005	76.65
2	0.006	0.002	0.002	0.001	66.15
3	0.002	0.002	0.002	0.005	54.6
4	0.006	0.002	0.006	0.001	51.45
5	0.002	0.006	0.006	0.001	82.1
6	0.006	0.006	0.002	0.005	52.6
7	0.002	0.006	0.002	0.001	61.95
8	0.006	0.006	0.006	0.005	57.75
9	0.002	0.002	0.002	0.001	71.57

In Plackett Burman method the importance of medium supplements can be studied. The advantage of using this method is that reduces cost, time saving and gives high ethanol yield [(4)] 9 trials have been done to estimate the concentration of ethanol to be added for high yield is given in table 1. The optimum values were found to be (g/l):$FeSO_4.7H_2O$ 0.002, $MgSO_4.7H_2O$ 0.001, $MnCl_2.4H_2O$ 0.006, and $ZnSO_4.7H_2O$ 0.006. The product concentration was found to be 82.1 g/l compared to conventional fermentation process.

Conclusions

Water hyacinth (*Eichhornia crassipes*) is a weedy lignocellulosic material which is used as a source for bioethanol production. Water hyacinth was pretreated with10% sulphuric acid. By this treatment water hyacinth which was composed of cellulose, hemicellulose and lignin were converted to simple sugars. But acid hydrolysis process produces some by-products like furfural, phenolic compounds etc. which are inhibitors for micro organisms involved in fermentation process. By this pretreatment technique the complex structures like **cellulose** (which is the highly branched sugar polymers) **hemicellulose** (which is the highly branched sugar polymers that contains sugar residues such as hexoses, pentoses and uronic acids) were converted to glucose and xylose respectively and **lignin** (complex, hydrophobic, cross-linked aromatic polymer in nature) was removed. Also by the enzyme hydrolysis the complex cellulose structure was broken into simple reducing sugars, ***glucose***, by the enzyme ***cellulase*** enzyme. Then the fermentation was carried out using ***Zymomonas mobilis CP4*** which converts the simple sugars into ethanol. The process like saccharification and fermentation were carried out in a single step called as ***simultaneous saccharification and fermentation (SSF)***, since it had reduced product inhibition. The ethanol content was estimated by pottasium dichromate assay.

The addition inducers can increases the yield of ethanol. This was screened by addition of four different inducers (**$FeSO_4.7H_2O$**, **$MnCl_2.4H_2O$, $ZnSO_4.7H_2O$ and $MgSO_4.7H_2O$)** at different concentrations using plankett- burmann design. We have carried out 9 different trials to find optimum concentrations of inducers to increase the ethanol concerntration. Further optimization studies are done to carry out fermentation in large scale.

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