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Comparative Study of Acid Pretreatment on Saccharification of Sawdust

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ABSTRACT

Lignocellulosic materials can be utilized for bioethanol production but its recalcitrant nature reduces its large scale utilization. The present study includes the comparative analysis and development of effective pretreatment process to break down sawdust which can releases a better amount of fermentable sugars. Three different acids Viz. H2SO4, HCl and Phenyl acetic acid were used for the pretreatments of sawdust followed by enzymatic hydrolysis with crude and pure form of cellulase. The amount of liberated sugars was measured by DNS method. Acid treatment with H2SO4, HCl and Phenyl acetic acid released 610 µg/mL, 650 µg/mL and 552 µg/mL of sugar respectively. Bioethanol production from saccharified sawdust was carried out by using Saccharomyces cerevisiae. Treatment with 0.1% w/v of pure cellulase enzyme to the acid treated sawdust can produced better amount of ethanol compared to crude cellulase and 0.01% w/v of pure cellulase.

KEYWORDS Lignocellulosic materials, Pretreatment, Saccharification, Bioethanol

Introduction:

Due to abundance of lignocellulosic material, its conversion to bioethanol is considered as one of the most important uses of cellulosic biomass as an energy source which also eliminates the utilization of fossil fuels and decreases problems related to our environment deterioration. The major obstacles in utilization of lignocellulosic materials are their recalcitrant nature and insufficient separation of lignin from cellulose that could further be converted to bioethanol and other useful chemicals. Various physical, chemical and biological pretreatment methods are known to break the lignocellulosic materials to release fermentable sugars. The choice of pretreatment process depends on the digestibility of cellulosic material, effect on environment and cost effectiveness.

Physical pretreatment mainly reduces the biomass size and crystallity, chemical treatment deals with the removal of lignin content by breaking recalcitrant nature of lignocellulosic materials to make easy accessibility of cellulose for microbial destruction while biological treatment uses various enzymes for hydrolysis process of cellulose. Separate chemical and enzymatic pretreatment found to be effective for saccharification and for the releasing of sugars.

Treatment with acid can be given by two different ways: (1) in the form of dilute acid and (2) in the form of concentrate acid. Treatment with concentrated acid is generally reported to give higher sugar yield (e.g. 90% of the practical glucose yield) and also higher ethanol yield compared to dilute acid treatment. Moreover, compared to dilute acid treatment, concentrated acid processes can operate at low temperature (e.g. 40 °C). The main problems associated with the utilization of concentrated form of acids are: (1) it requires expensive alloys or specialized non metallic constructions like ceramic or carbon brick lining because of its corrosive nature (2) acid recovery is energy demanding process (3) high investment and maintenance cost (4) production of inhibitory compounds etc.

Dilute acid hydrolysis method is most commonly applied to hydrolyse lignocellulosic materials. The first established dilute acid hydrolysis process was the Scholler process (Faith, 1945). This process was a batch process in which wood material was kept in 0.5% H_2SO_4 at 11-12 bars of pressure for 45 minutes. Recently, dilute acid hydrolysis are performed in a batch mode with retention time of few minutes.

Dilute or concentrated form of acid mainly solubilizes the hemicelluloses fraction for easy accessibility of cellulose to enzymatic hydrolysis. Generally dilute acid treatment is preferred for bioethanol production. Dilute acid treatment requires high temperature and pressure for effective saccharification. Treatment to the branches and leaves of olive tree with dilute H_2SO_4 releases 48.6% sugars at 170 °C (Cara ***et al***., 2008). Strong acid treatment provides flexibility for feed stock selection and gives high monomeric yield of sugars and requires moderate temperature of 70 - 121 °C.

Method & Materials:

Raw material collection: Sawdust was collected from Sharda Vijay saw mill, Maroli, Gujarat. Collected sawdust was brought to the laboratory and sieved to get fine powder. Sawdust was washed with tap water and dried in oven at 110 °C before pretreatment with different acids.

Production of cellulase: Crude cellulase enzyme was produced by ***Bacillus licheniformis*** isolated from the gut of termite. For the cellulase production CMCase media which consist of (g/L): $NaNO_3$- 2.0 g, K_2HPO_4- 1.0 g, $MgSO_4$- 0.5 g, KCl- 0.5 g, Peptone- 2.0 g, Carboxy Methyl Cellulose- 10 g was used and pH was set to 6.5. 5% inoculum was utilized for the production and flask was incubated at 120 rpm and at 30 °C for 48 h.

Pretreatment by various acid: Oven dried sawdust was treated by three different acids (H_2SO_4, HCl, phenyl acetic acid) using different concentration like 0.1% v/v, 1.0% v/v and 5.0% v/v. Phenyl acetic acid was dissolved in sterile water by heat treatment prior to sawdust hydrolysis. For the treatment purpose, the ratio of different chemicals and sawdust was selected to 20 mL/ 1 g i.e. 5% sawdust was added to respective acid solutions. Treatment was given for 2 h at 60 °C and at 120 rpm.

Neutralization process: After treatment was over, the pH of treated sawdust was adjusted to 7.0 by repeated washing under running tap water. Again it was dried in oven at 110 °C for 24 h.

Enzymatic treatment: Dried pretreated sawdust was exposed to crude cellulase enzyme produced by ***B. licheniformis***. Similar enzymatic treatment was given by using 0.01% w/v and 0.1% w/v pure form of cellulase which have concentration of 10 U/mL. For the enzymatic treatment 10% chemically treated sawdust was used and soaked in enzyme preparation for 2 h at 30 °C.

Sugar estimation: The liberated sugar after chemical and enzymatic treatment was measured using DNS method given by Miller (1959).

Ethanol production: Ethanol production was carried out by treating sawdust with different acids and then with crude cellulase enzyme by using ***Saccharomyces cerevisiae***. Ethanol production was estimated by iodometric method (Experimental Microbiology).

Result & Discussion:

Cellulase production: After 48 h of incubation, isolated strain of ***B. licheniformis*** from termite gut was shown to produce cellulase enzyme which have 310 µg/mL of CMCase activity.

Pretreatment by various acids:

No.	Chemicals	Chemical Conc. (%)	Sugar Conc. (µg/mL)	Ethanol Conc. (mg/mL)
1		0.1	500	
2	H_2SO_4	1.0	510	17.6
3		5.0	610	
4		0.1	560	
5	HCl	1.0	460	20.0
6		5.0	650	
7		0.1	460	
8	Phenylacetic acid	1.0	462	15.8
9		5.0	552	

Table-1: Pretreatment by various acids and 0.1% w/v pure cellulase

No.	Chemicals	Chemical Conc. (%)	Sugar Conc. (µg/mL)	Ethanol Conc. **(mg/mL)**
1	H_2SO_4	0.1	160	8.0
2		1.0	202	
3		5.0	258	
4	HCl	0.1	196	8.4
5		1.0	224	
6		5.0	260	
7	Phenylacetic acid	0.1	146	6.0
8		1.0	200	
9		5.0	240	

Table-2: Pretreatment by various acids and 0.01% v/v pure cellulase

No.	Chemicals	Chemical Conc. (%)	Sugar Conc. (µg/mL)	Ethanol Conc. (mg/mL)
1	H_2SO_4	0.1	480	15.4
2		1.0	460	
3		5.0	500	
4	HCl	0.1	490	17.8
5		1.0	600	
6		5.0	620	
7	Phenyl acetic acid	0.1	430	13.8
8		1.0	450	
9		5.0	480	

Table-3: Pretreatment by various acids and crude cellulase

Treatment using H_2SO_4, HCl and phenylacetic acid along with 0.1% w/v of pure cellulase, 0.01% w/v of pure cellulase and also by crude cellulase enzyme to the sawdust, released considerable amount of fermentable sugars. Fermentation of liberated sugars with the help of ***Saccharomyces cerevisiae*** can produced the highest amount of ethanol i.e. 17.6 mg/mL, 20.0 mg/mL and 15.8 mg/mL respectively when sawdust was treated with H_2SO_4, HCl and phenylacetic acid along with 0.1% w/v of pure cellulase enzyme.

Conclusion:

Lignocellulosic materials are utilized for the production of bioethanol as an alternative and cheap source to starchy materials which would otherwise expensive, utilize crop land, and require more input of fertilizer, pesticide and energy. The production rate of bioethanol is dependent on effective hydrolysis and saccharification of lignocellulosic materials. So, effective treatment must be developed to saccharify lignocellulosic materials for liberating maximum amount of fermentable sugars. Pretreatment to sawdust with different acids liberated considerable amount of sugars followed by enzymatic treatment. HCl was known to produced the highest amount of fermentable sugar compared to H_2SO_4 and phenylacetic acid. Fermentation using ***saccharomyces cerevisiae*** can produced maximum amount of ethanol in the successive treatment to sawdust by H_2SO_4, HCl and phenylacetic acid along with 0.1% w/v of pure cellulase enzyme. Crude cellulase enzyme produced by the termite gut bacterial isolates also released better amount of sugars and were responsible for the production of good content of ethanol which was higher than the treatment with different acids along with 0.01% w/v of pure cellulase but was less than that of acidic treatment along with the treatment of 0.1% w/v of pure cellulase. Utilization of crude form of cellulases minimizes the cost of ethanol if suitable and cheap substrate were used. By changing the parameters of cellulase production and also by genetic modification of producer organisms, better amount of cellulases can be produced which will saccharify the large amount of complex cellulosic material to produce considerable amount of bioethanol.

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