Bio-Ethanol Production from Cornstalk Containing Wastewater

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Abstract: Having identified poorly efficient pre-treatment methods as the rate limiting step to cellulosic ethanol production, combination of two or more pre-treatment methods are being examined. In this study, three stage alkaline-acid-enzyme pre-treatment was attempted in addition to using waste water containing organic matter. Optimum Reducing Sugar yield of 604.96mg/g cornstalk was obtained when 10% (w/v) NaOH and 5% (v/v) H_2SO_4 was used in succession at 120^0C for 60 minutes. The use of wastewater containing cornstalk for the production of cellulosic ethanol was found to have desirable effects on Reducing Sugar yields. Total Reducing Sugar yield was increased to 874.05mg/g cornstalk and fermentation efficiency of 89.44% for wastewater containing 40g/L cornstalk.

Keywords: Cellulosic ethanol, cornstalk, fermentation, pre-treatment.

1. INTRODUCTION

Renewable energy has been able to compete effectively with fossil fuels in areas such as global warming and recyclability. However, pollution, ecosystem alterations and plenteous fresh water requirement remains common to both energy generating options.

Biomass such as Corn stalks and cobs, Sunflower, and Switch grass take up large volumes of water for growth, processing and conversion to bio-fuel. With most advanced economies of the world running bio-fuel and fossil fuel production concurrently, there is an alarming stress on the available water resources. Water footprint as part of sustainable bio-fuel production, is a growing source of concern for bio-fuel producing plants, activists and societies [1, 2].

Within the past decade, water consumption has significantly reduced from as much as 6 gallons of water in 1994, to as low as 3 gallons of water per gallon of dry grind ethanol produced in 2010 [3]. However, cellulosic ethanol plants are estimated to still consume around 6 to 10 gallons of water per gallon of ethanol [3]. Water coming in direct contact with the biomass being processed is known as process water. Non-process water includes the water which does not directly come into contact with the feedstock such as water circulating in cooling towers, boilers, heat exchangers and water going into the Reverse Osmosis (RO) unit. The step that demands the most amount of process water is the pre-treatment step. This step includes soaking and the chemical methods being applied to distort lignin and hemicellulose structure for enhanced cellulose hydrolysis. Researches to optimize the conversion process are underway with emphasis on increasing ethanol yield [4].

This study was aimed at reducing the amount of fresh water used as process water. The objective of this study was to investigate the effects of municipal waste water on

- Hydrolysis of corn stalk during pre-treatment
- Quantity of ethanol produced during fermentation.

2. MATERIALS AND METHODS

2.1. Substrate and Chemicals

Corn stalk and leaves harvested in the Fall of 2013 from a farm in the Cukurova region of Turkey, was used as biomass feedstock. The material was washed and oven dried at 105^0C for 96 hours and ground with Spex sample prep 8000 M/mixer mill. The ground material was then passed through a 6mm mechanical sieve as this was the minimum pore size through which ground corn stalk could pass through. Fractions that passed through the sieve were collected and stored.

Cornstalk was characterized for humidity, lignin, ash content [5], cellulose [6], holocellulose and hemicellulose [7]. Chemical analysis for total carbohydrates (TCH) and Total Reducing Sugars (TRS) were conducted. These were done, respectively, using methods described by Rao and Pattabiraman [8] and the dinitrosalicylic acid (DNS) method proposed by Miller [9]. All the chemicals used were of analytical grade and they were obtained from Sigma and Merck.

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Table 1: Composition of Untreated Corn Stalk

Structural Composition							
Constituents	Cellulose	Hemicellulose	Holocellulose	Lignin	Ash	Moisture content	Others
Composition (%)	33.09	21.90	54.99	15.45	9.62	6.1	13.84
Chemical Composition							
Constituents	TRS		TCH				
Composition (mg/g corn stalk)	40.26		68.83				

2.2. Pre-treatment

The biomass feedstock, Corn stalk was subjected to a combined alkaline-acid-enzyme pre-treatment process. Sodium hydroxide (10% w/v) was first applied at a 120^{0}C for 60minutes after which Sulfuric acid (5% v/v) was applied. The second stage was also conducted for a retention time of 60minutes at a 120^{0}C. The resulting mixture was then filtered and washed before proceeding to treat with Cellulase at a concentration of 25U per gram of Corn stalk

This was conducted using waste water containing 30, 40 and 50g/L Corn stalk. A control experiment using distilled water was also conducted.

2.3. Fermentation to Ethanol

Yeast growing medium was prepared using methods described by Anwar *et al.* [10]. After sterilization of the medium, 1g of active dry yeast (*Saccharomyces cervisiae*) was added and incubated by placing in a water bath for 23h at 30^{o}C.

The pre-treated Corn stalk media having been filtered and the pH of the filtrate adjusted to 4.25 using 0.1 % H_2SO_4, was analyzed for TCH and TRS yield. This was followed closely by inoculation with yeast growing medium with volume of inoculate being 7% of substrate volume.

The conical flasks containing inoculated samples and control experiments were then sealed using cotton wool and parafilm and placed in a water bath maintained at a temperature of 28^{o}C. Samples were withdrawn at 0, 2, 4, 6, 8, 24, 36, and 48h.

The withdrawn samples were analyzed for Ethanol using the Potassium dichromate method, [11] Total reducing sugars, total carbohydrates, pH.

3. RESULTS AND DISCUSSION

3.1. Composition of Cornstalk

In Table **1**, the structural composition of corn stalk is represented as percentage content of constituents on a wet weight basis. The TRS and TCH content is also represented in mg/g of corn stalk. The major constituents of ligno-cellulosic biomass required for the production of cellulosic ethanol are cellulose and hemicellulose. These constituents are broken down to monosugars and fermented to bio-ethanol. However, due to the cell wall structure of fibre crops, co-product recovery and downstream enzyme inhibition, the knowledge of lignin content has become as important as holocellulose content. The knowledge of the initial TRS and TCH content is particularly important because it is required to evaluate the effectiveness of pre-treatment and hydrolysis methods as well as the efficiency of the fermentation process.

3.2. Reducing Sugar yield from Cornstalk Containing Wastewater

TRS yield from wastewater containing 30, 40 and 50g/L cornstalk was found to be very high in comparison to TRS yield from 1g/L substrate loading. TRS yields increased from 604.96mg/g cornstalk to as high as 874.05mg/g cornstalk. However, TRS yield dropped to 559.80mg/g cornstalk when wastewater containing 50g/L cornstalk was pre-treated. This could be due to low chemical to substrate ratio, low oxygen percolation, substrate clustering as well as higher possibility of microbial contamination. Table **2** shows the various TRS yield from a 1g/L substrate loading and 30, 40, 50 g/L cornstalk containing waste water. The results prove that to a certain degree, although efficiency of pretreatment process depends mainly on biomass, its structure and lignin content [12], waste water containing organic matter can be beneficial to monosugar production from lignocellulosic biomass.

3.3. Ethanol Production

Ethanol concentration increased slowly through to the 6th hour after which sharp decrease in concentration was observed. According to Klinke *et al*., [13] Phenols from Lignin degradation generally inhibit yeast growth and ethanol production rate but not ethanol yield. The decrease after the 6th hour of fermentation time was probably, due to the

Table 2: TRS Yield from Wastewater Containing Cornstalk and non Waste Water Containing 1g/L Cornstalk

	Distilled Water	Wastewater		
Cornstalk concentration (g/L)	1	30	40	50
TRS (mg/g cornstalk)	604.96	762.74	874.05	559.80

consumption of accummulated ethanol by the microbes. It has been observed that when ethanol builds up in the medium, the microbial community adapts to simulteneously use up both ethanol and the sugars. But after adaptation to the ethanolic environment, the microbes begin to grow again [14]. As can be seen from Figure **1**, ethanol volume in the fermentation medium increased sharply after the 8th hour and began to stabilize as it approached the 36th hour. By the 36th hour of fermentation time, ethanol yield reached close to 50% with maximum ethanol concentration of 15.89g/L being recorded for wastewater containing 40g corn stalk/L. There was however, a gradual reduction in ethanol fraction towards the 48th hour of fermentation time. The completion of the microbial life cycle or the exhaustion of monosugars are probable causes for the gradual decrease in ethanol concentration. As can be seen from Figure **2**, pH dropped to as low as 2.02 from 4.25 in some of the mediums, this acidic environment could also have affected the production of ethanol. The acidic medium can be attributed to increased CO_2 in the system. Figure **3** is a graph showing the depletion of TRS concentration in the course of fermentation. This is important because, it depicts the effectiveness of sugar transporters of *S. cerevisiae* cells at translocating different sugars across the cell membrane and general efficiency of the fermentation process. As can be seen in Figure **3**, consumption of TRS was a bit slow within the first 6hrs decreasing at an average rate of 2000mg/hr. This could be due to the presence of high concentration of TRS as extremely high TRS concentrations have been found to inhibit fermentation process. Another plausible reason for a slow TRS depletion could be the consumption of both TRS and ethanol by the microbes as an adaptability technique. TRS consumption rate however, increased considerably within the next 18hrs with TRS concentration clearly approaching zero. Overall efficiency of fermentation process with respect to yeast cell efficiency, ethanol yields, ethanol production efficiency and TRS values were calculated and summarised in Table **3**.

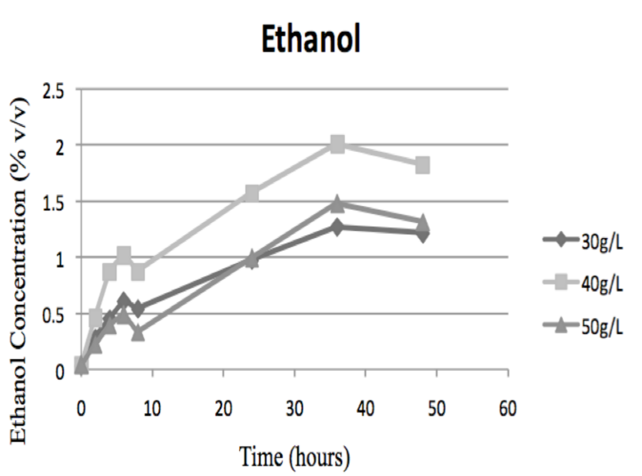

Figure 1: Ethanol produced from corn stalk.

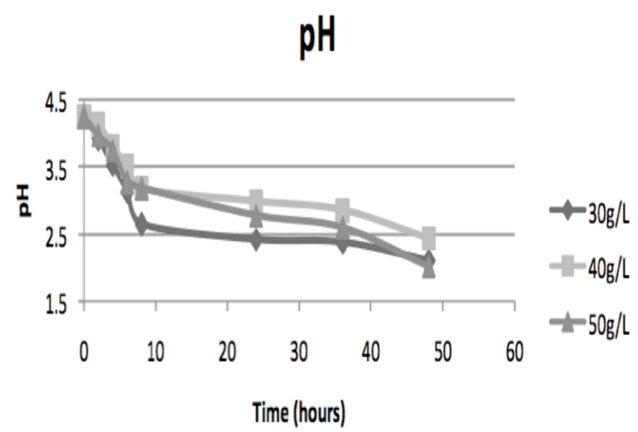

Figure 2: pH of Corn Stalk fermentation medium.

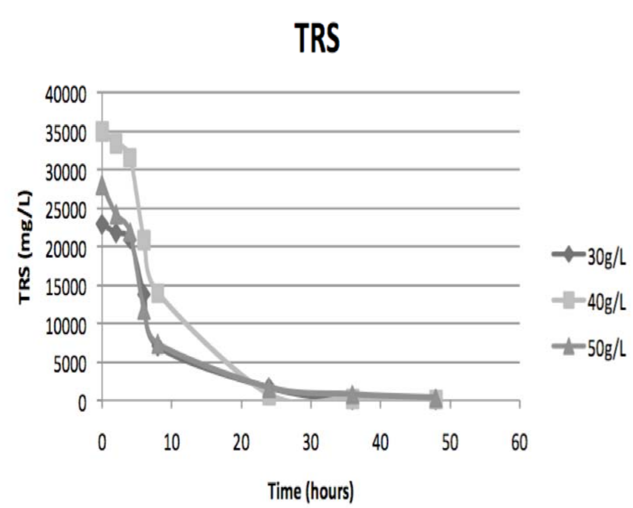

Figure 3: Reducing Sugars concentration during fermentation.

Table 3: Summary of Efficiency Fermentation Process

Initial corn stalk concentration: Ch,0 (g/L)	30	40	50
Maximum Ethanol Volume: Emax (%vol)	1.27	2.01	1.48
Initial TRS: Cs,0 (g/L)	22.88	34,96	27,99
Maximum Ethanol Concentration: Ce,max (g/L)	10.04	15.89	11.67
Time of Max. Ethanol Conc.: tmax (hrs)	36	36	36
TRS at tmax: Cs,max (g/L)	0.025	0.19	0.74
Max. Ethanol Amount: Ethmax (g/gcornstalk)	0.33	0.40	0.23
Rate of Ethanol Production (g/L.h)	0.28	0.44	0.32
Yield of Ethanol of Yeast (%)	85.93	89.44	83.79
Yield of Ethanol (%)	43.86	45.45	41.68

3. CONCLUSION

The use of wastewater containing cornstalk for the production of cellulosic ethanol was found to not only improve TRS yield, but to also reduce fresh water requirement for lignocellulosic pre-treatment. TRS yields was increased to 874.05mg/g cornstalk and fermentation efficiencies of 89.44% for wastewater containing 40g/L cornstalk. TRS yield was found to decline greatly as for waste water containing organic matter concentration above 40g/L indicating that Organic matter concentration is an important factor.

Combination of two or more primary pre-treatment methods and water sourcing from organic matter containing supplies can go a long way in pushing cellulosic ethanol production further up the ladder of development. Depending on the source and organic content, non-fresh water sources can be used for cellulosic ethanol production and desirable results will be obtained.

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