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FERMENTABLE SUGAR PRODUCTION AND SEPARATION FROM WATER HYACINTH USING ENZYMATIC HYDROLYSIS

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Abstract- *Water hyacinth containing a remarkable amount of cellulose which is found throughout the world as unusable material that can be used as one of the promising source for the production of glucose, initial step to produce bio ethanol. In this current study different types of treatments highest glucose concentration was obtained by hot water treated method. In addition, glucose was produced from water hyacinth using cellulytic enzyme pseudomonas sp., isolated from cow dung. Glucose concentration and production rate increases with the increasing of substrate concentration and enzyme loading, particle size 45µm and a pH 6.00 and temperature 40 ^oC are the optimum for glucose production. A kinetic model rate expression has been developed for enzymatic hydrolysis of water hyacinth based on the Michaelis – Mentens model and parameters are determined. 0.15gm Reducing sugar are separated from the mixture of glucose water solution using 2 gm of water hyacinth. 0.531mg/l Cellulytic composition in water hyacinth are determined.*

Keywords: Hydrolysis, Water hyacinth, Pseudomonas sp., Michaelis – Mentens, Kinetic model

1. INTRODUCTION

Energy requirements increasing day by day all over the world and oil, gas, crude oil etc. are the considerable weapon to fulfill the growing demand throughout the world. But infarct, that not going to possible to fulfill required the demand by these sources [1, 2]. For these growing demand of fuel, worlds every country are trying to find out the other way and among them renewable energy such as solar energy, biodiesel production from biomass, bio ethanol production from biomass etc. are the most appropriate outcome. Lignocellulosic biomass presents an attractive substrate for bio ethanol production. Ethanol production based on corn[3], wheat[4]and sugarcane, lingo cellulosic biomass can be found in residues from the agriculture and forest industry or lignocellulosic rich energy crops, such as switch grass and elephant grass, can be grown on marginal land unfit for cultivation of crops for human or animal consumption[5] and researchers converted many lignocellulosic materials in to fermentable sugar initial step to bioethanol[6].In the present work water hyacinth was used for the production of fermentable sugar initial step to produce ethanol. Water hyacinth, has contain about 20% of cellulose, 10%lignin, and 33% hemicelluloses, has been marked as the world's worst water weed and has garnered increasing international attention as an invasive species. Water hyacinth has been identified by the International Union for Conservation of Nature (IUCN) as one of the 100 most aggressive invasive species [7] and recognized as one of the top 10 worst weeds in the world [8]. So this high content of cellulose got strong favorability to the worlds scientists for sugar production. The conversion of lignocellulosic materials to bioethanol needs some important issues may be difficult for many situations [9]. Processing of lignocellulosic biomass to ethanol consists of four major unit operation; pretreatment, Hydrolysis, fermentation and product separation or purification [10]. In this work we produced fermentable sugar which is the initial step to produce bioethanol by using first two step noted above followed by separation. Generally two basic conventional approaches are followed for converting biomass into fermentable sugars. Saccharification can be carried by acid or enzymatic hydrolysis. Enzymatic hydrolysis got its favorability than acid hydrolysis for many reasons [10]. But still their some factors should be considered as a headache for the production of fermentable sugar such as making much accessible cellulose more to enzyme, Degree of

polymerization, pretreatment severity etc. There are many goal of the work among them enzymatic hydrolysis gets highest priority and study the activity of the enzyme at different conditions. Separating fermentable sugar and developing Michaelis–Mentens model for the designing of an effective process for the production of final product.

2. MATERIALS AND METHODS:

2.1 Substrate Preparation:

Water hyacinth were collected from the nearby source and washed with water to remove the dust or other dirty. After that WH dried at 105 ^{O}C for 5-6 hours in the drier oven.

2.2 Pretreatment of Substrate:

Pretreatment of lignocellulosics aims to decrease crystallinity of cellulose, increase biomass surface area, remove hemicellulose, and break the lignin barrier [8]. Substrate are pretreated physically and chemically to accessible the substrate into enzyme. There different types of treatment among them those are applied in this work are given below:

(a) Hot water Treated: 10gm of dried water hyacinth were measured and mixed with 300ml distilled water in a biker and heated to the boiling point for 15 minutes. Treated WH dried at 102-105 ^{O}C for 3 hours and was blended to get the desired size of the particle.

(b) H_2SO_4 treated: 20gm of dried water hyacinth were measured and mixed with 150 ml of 1 %(v/v) of H_2SO_4 solution and heated at 115^{O}C in oven for 3 hour. After that, it was washed with continuous charge of distilled water until the neutralization of water hyacinth sample and dried in air dryer for 3 hours at 105^{O}C and was blended to get the desired particle size.

(c) NaOH treated: 10gm of dried water hyacinth were measured and mixed with 100 ml of 1 %(w/v) of sodium hydroxide solution and heated at 112^{O}C in oven for 3 hours. Then washed again with distilled water to neutralize the sample and dried in air dryer for 3 hours and blended and particles with desired size were used for further experiment.

(d) NH_4OH treated:10gm of dried water hyacinth were measured and mixed with vigorously in 100 ml of 1 %(v/v) of ammonium hydroxide solution followed by heating in oven at 110^{O}C for 2 hour. Then neutralization was done by distilled water and dried in air dryer for 3 hours and blended into particles of desired size.

2.3 Hydrolysis:

The hydrolysis of pre-treated **water hyacinth** was performed in rotary flasks shaker (Model: LRD: 750) with working volume of 150mL in 250mL flasks. Various amount of treated water hyacinth was taken a in each flask prior to the experiment and treated with Sodium acetate buffer (0.05 M) to maintain the p^{H} of the hydrolysis environment. All the components and p^{H} were assumed to have a uniform distribution in the flask due to continual rotation. All experimental run were conducted with addition of various predetermined amount of enzyme in the degradation environment with different amount of water hyacinth, different size of water hyacinth, different enzyme loading, different pH and different temperature to delineate the corresponding respect . Samples were taken at every 1 hour interval; boiled for 5 min to destroy the enzyme, thus confirming the ceasing of the reaction. Then the samples were centrifuged, and analyzed for glucose concentration in **UV**-spectrophotometer according to the method by Miller [9].

3. RESULT AND DISCUSSION:

3.1 Effect of substrate pretreatment

Pretreatment is considered one of the most crucial steps in bio ethanol production since it has a large impact on all other steps in the conversion process. Different types of treatments were used to increase the substrate activity to produce reducing sugar. mineral acid such as sulfuric acid ,base treatment such as sodium hydroxide, ammonium hydroxide and hydraulic treatment such as cold water or hot water treatment. Among them highest concentration was observed for hot water treatment.

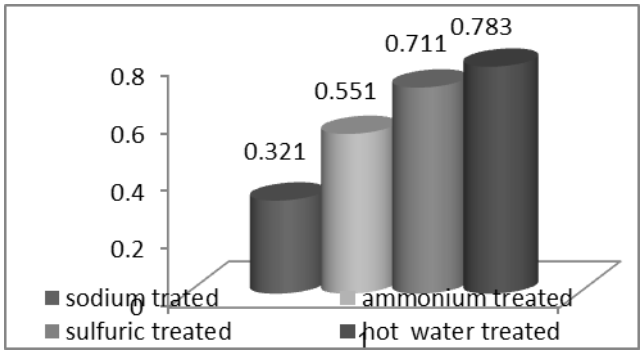

Figure 1: Effect of various types of pretreatment used for substrate (pH: 5.5 (acetate buffer), water hyacinth: 5 gm/L, enzyme: 15ml/ 1L mixture) on glucose yield

3.2 Effect of Substrate Particle Size

An efficient conversion of lignocelluloses into fermentable sugars is a key step in producing bioethanol in a cost effective and environmentally friendly way. Different sizes of particle ranging from 45 micrometer to 500 micrometer were taken for particle effect. Among them 45 micrometer shows greater production of reducing sugar.

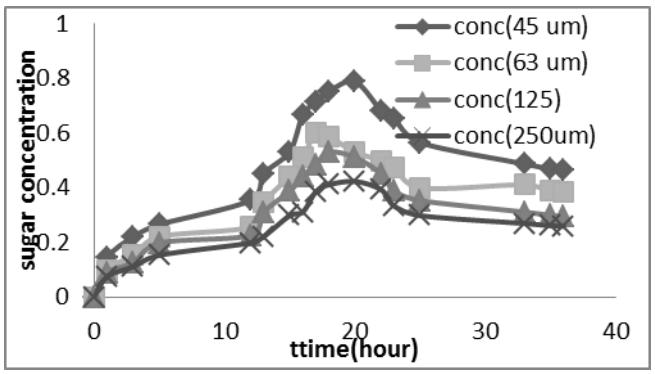

Figure 2: Effect of particle size on sugar production (water hyacinth: 3.3gm/l, enzyme: 30ml/l, pH: 5.5)

3.3 Effect of Enzyme loading

1ml to 18 ml enzyme was used to observe the effect of enzyme loading. Among them lower concentration was observed for 1ml enzyme and higher for 18 ml enzyme. It shows higher concentration of glucose for higher enzyme loading. This may be due to higher enzyme-substrate-active complex formation due to increased active sites by both charges

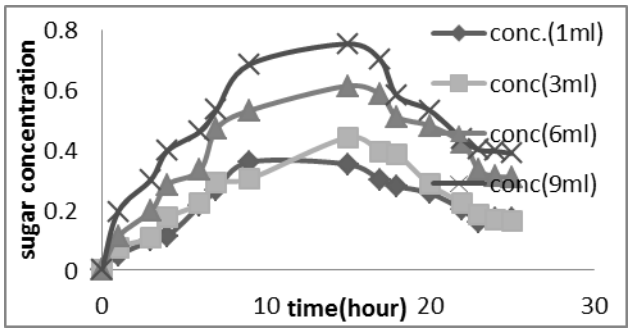

Figure 3: Effect of Enzyme loading (pH: 5.4 &wh loading: 3.33gm/L, temp: 24)

3.4 Effect of Substrate Loading

0.25mg to 1.5gm substrate was used to observe the effect of substrate. Among them lower concentration was observed for 25mg substrate and higher for 1.5gm substrate The effect of substrate concentration using water hyacinth is shown in figure 4 given below. In this observation it shows that the concentration of the glucose production increases with increasing of substrate concentration.

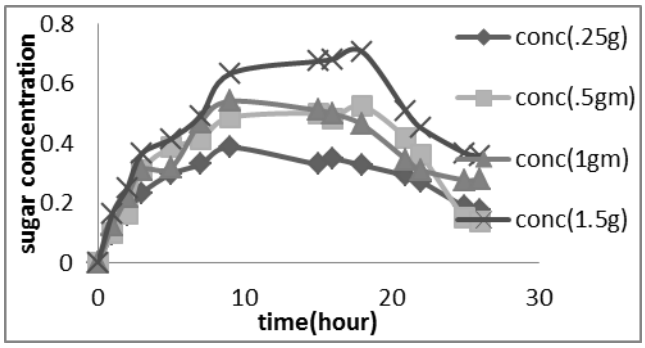

Figure 4: Effect of initial substrate concentration on sugar production (pH: 5.5, temperature: 26, enzyme: 30 ml/L)

3.5 Effect of Temperature

Figure 5 represents the outcome of the consequence of temperature on the extent of bioconversion of glucose production where, the alteration of enzymatic efficiency of pseudomonas sp. for glucose production with different temperature is showed and the optimum temperature of 40±2°C was required to attain the most excellent water hyacinth conversion to glucose

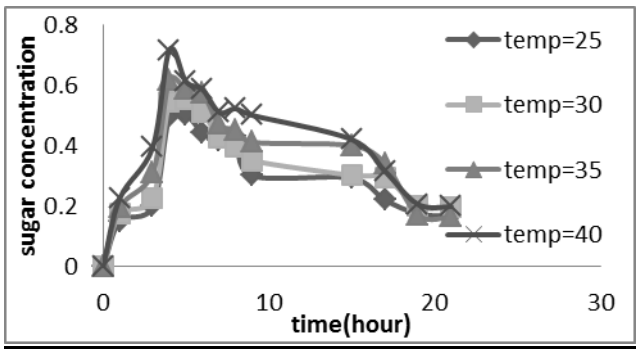

Figure 5: Effect of temperature on sugar production (water hyacinth loading: 3.33gm/L, pH: 5.5, enzyme loading: 30 ml/L).

3.6: Effect of pH

The pH effect for the activity of cellulytic bacteria from ranging 5 to 8 were observed the outcome of pH alteration on glucose liberation is shown I Figure 6. The pH range between 6.0±0.2 gave the optimum yield of glucose

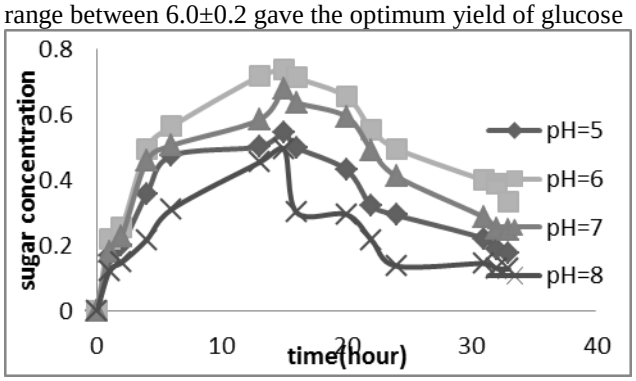

Figure 6: effect of pH on sugar production (enzyme loading 36ml/L, WH loading: 3.33gm/L, temperature: 25 ± 2 ^{O}C).

4. DEVELOPMENT OF KINETIC MODEL:

A typical kinetic results by graphical Differentiation of curves using the initial rates method, afforded the plot of initial rates at different levels of substrate initial concentrations as shown in Figure 7.

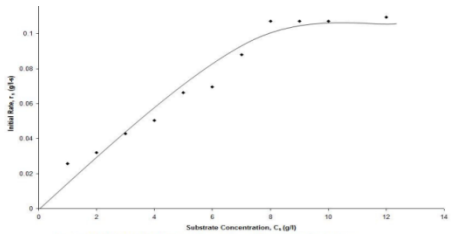

Figure 7: The initial rate as a function of substrate concentration (a typical curve).

From this curve, it can be observed that the reaction rate is proportional to the substrate concentration (that is, first order reaction) when the substrate concentration is in low range is also evident that the rate of reaction move toward a steady value as the substrate concentration becomes elevated. So we can say, the reaction rate alters progressively from first order to zero order as the substrate concentration was amplified. This form of conduct is

commonly described by the Michaelis-Menten kinetic expression such as:

$$V_p = \frac{V_{max}[S]_o}{K_m + [S]_o} \text{.....(1)}$$

Where, V_{max} (the maximum reaction rate) and K_m (rate constant) are the kinetic parameters, which are needed to be experimentally determined and $[S]_o$ is substrate concentration

Applying the Line weaver – Burk method to linearize the rate expression by inverting equation (1) yields:

$$\frac{1}{V_p} = \frac{1}{K_m} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]_o} \text{..... (2)}$$

So, from this straight line plot, the corresponding parameters (K_m & V_{max}) can be determined from intercept $1/V_{max}$ and the slope K_m/V_{max}.

The equation (2) is plotted by the data obtained with differentiation of initial glucose production by time for various initial substrate concentrations.

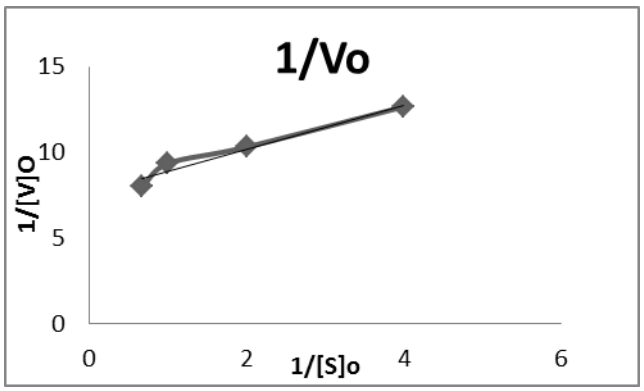

Figure 8: Linearized kinetic modeling plot.

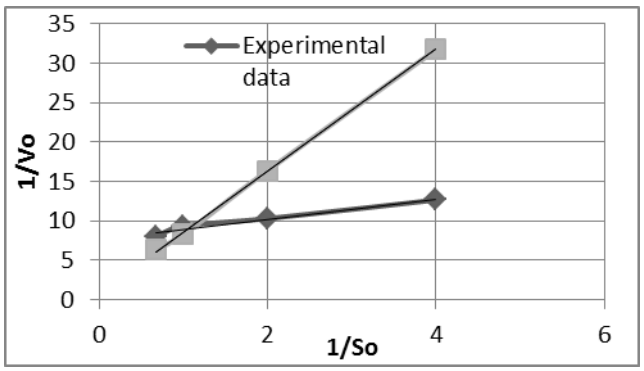

Figure 9: comparison between theoretical and experimental data.

The data best fits a straight line with slope is 1.284 and intercept is 7.617 from this plot the kinetic parameters (V_{max} and K_m) were estimated as 0.102 gm/ (L. hour) and 0.778 gm/L respectively.

Based on the evaluated kinetic parameters, the model equation is given as:

$$V_o = \frac{0.778[S]_o}{5.932 + [S]_o}$$

5. CONCLUSION:

Pretreatment is an important step to make cellulose more accessible to the enzyme that converts the carbohydrate polymers into fermentable sugar. In maximum time among different pretreatment acid pretreatment are used such as The acid pretreatment and enzymatic hydrolysis were used to evaluate to produce more sugar, to be fermented to ethanol But we have observed different acid pretreatment such as sulfuric acid,, base pretreatment such as ammonium oxide, sodium hydroxide and hydrothermal pretreatment such as hot water(at 105 for 15 minutes).We have highest result in hot water treatment among all other treatment and these treatment are better than other in many cases such as it is regarded as safer-equipment corrosion is reduced- and more environmentally friendly, as often no chemicals are required .

Another mentionable work we have done; the hydrolysis process were done by isolating the cellulytic bacteria from cow dung and separated the highest activity shower cellulytic enzyme *pseudomonas sp.* But in many research that was not identified such as Separated hydrolysis and fermentation of water hyacinth leaves for ethanol production; in these case different type of enzyme were collected from chemical storage named Sumitime C; Shin Nihon Chemical Co. Ltd., Japan to hydrolyze the cellulose. In this study fermentable sugar are separated and testified for sugar which is the unique work done. This is very important to know that how much fermentable sugar can be possible to produce using how amount water hyacinth whether for producing bioethanol or others work.

Another unique work done by measuring the cellulose concentration by passing of time; It should be noted that cellulytic concentration has strongly proof on behalf of the production of the fermentable sugar. Kinematic study has done and unknown parameters are calculated for designing the more economical scarification process and respectively for bioethanol production. It can be concluded that pre-treatment of water hyacinth enhances the rate of glucose production, while particle size of 45μm was found to be more favorable among the analyzed sizes. Operating temperature of 40^OC and pH of 6.0 gave the best activity within the range of time investigated

The kinetics parameters of the reaction were obtained as V_{max} = 0.778 gm/ (L. hour) and K_m =5.932 gm/L respectively.

7. ACKNOWLEDGEMENT

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