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The Production of Ethanol from Sugar Beet Waste by Immobilized Saccharomyces Cerevisiae

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Abstract

Saccharomyces Cerevisiae cells were immobilized in calcium alginate beads and activated charcoal for use in the production of ethanol from batch fermentation of sugar beet waste. Treatment of the waste with NaOH to increase the ability of lignocellulose material to hydrolysis by acid (2N H_2SO_4) to monosaccharide and disaccharide (mainly glucos). The high reducing sugar concentration obtained was equal to 9.2gm/100ml (10Brix) after treatment. Fermentation parameters, are (pH, glucose concentration (2.5-25 gm/100ml), immobilized agent concentration (2.5-25 gm/100ml) were studied to find the optimum physiological condition. And the highest ethanol concentration obtained from the fermentation in the presence of 20%(wt/v) calcium alginate was (9.322%(wt/v)) at 13.75%(wt/v) glucose concentration and pH 5 .The experimental results were correlated by empirical second order polynomial equation with correlation coefficient 96.734% and variance 93.574%

***Keywords*:** sugar beets, ethanol.

Introduction

Bioethanol is ethanol (C_2H_5OH) a colourless liquid with a faint odour [1]. It was produced by biological fermentation of biomass. Biomass means vegetable and animal substances consisting of the biodegradable fraction of products wastes and residues from agriculture, forestry and related activities, or industrial and municipal waste [2]. The production of ethanol is a world wide industry .It is not only used as an alcoholic spirit but also as pure product in food, pharmaceutical personal care detergents, adhesives, lubricants, it is also used as an intermediate material to produce various products and other industries. A large scale application of bioethanol is it's use as a transportation fuel. When Henry Ford in 1907 re-introduced to American motoring public by producing his first vehicle to run on ethanol [3].

Bioethanol can be produced from a range of agriculture feed stocks, such as starch crops, sugar crops, woody crops and sugar industry bioproducts such as sugar cane bagasse, and sugar beet waste [4].

In the United States, approximately 6.8 billion liters of ethanol were manufactured in1997 from starch . Brazil produced 14 billion liters of ethanol from sugar cane in 1997. Other countries now developing bioethanol industries include Spain, France and Sweden in the EU; as well as Australia and Canada [5].

The Advantage of using sugar beet waste as a substrate for bioconversion are that it has a high carbohydrate content (cellulose 40-50%, and 20-30% hemicellulose) and a cheap substrate(waste product) Cellulose ,a polymer of C6 sugar (glucose) ,Hemicellulose aco-polymer consisting of C5 sugar (mainly xylose)and Lignin a "random " polymer consisting of mainly are shown in figure 1. Both the cellulose and hemicellulose fraction can be fermented to ethanol after a suitable pre-treatment and hydrolysis steps. The pre-treatment of lignocellulose was necessary for the separation of lignin from structure, usually by steam explosion, dilute acid and dilute alkali, followed by hydrolysis with acid, enzymatic by fungus (*Trichoderma viride*) and thermochemical process [6].

The production of ethanol via anaerobic yeast growth may be achieved by using either free or immobilized cell systems, the immobilized yeast-cell system can be defined as any system in which yeast cells are confined within a bioreactor [7].The principle method, for immobilization were: crosslinking, entrapment, covalent binding, encapsulation and adsorption. The advantage of using immobilized cells were provides of much greater cell concentration, higher conversion of glucose to ethanol and reducing the contamination of the product [8].

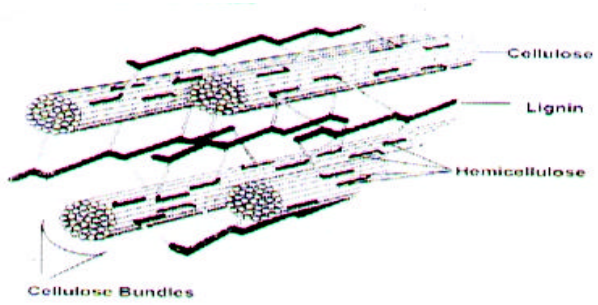


Figure (1)Structure of lignocellulose[9]

Experimental Work

Organisms

Baker`s yeast (*Saccharomyces Cerevisiae*) of trade mark name YuVa (Turkey) was used in this research .

Raw Materials

Sugar beet waste (SBW) which is produced as of sugar manufacture was used as a basic substrate in all experiments. It was kindly provided in the form of small dry clinders by the sugar factory in Mosul / Iraq.

Analysis of sugar beet waste showed the following constituents (%) taken from the factory . Moisture 8.59%,Disccharide (sucrose) 3.5%,Monsaccharide (glucose) 0.7%,Cellulose 38.6%,Hemicellulose 26.5%,Lignin 12.1%,Protein 8.5%,Ash 2.8% .

Ethanol Production Media

The media was prepared as follows for one-liter of (SBW) extract with certain glucose concentration

$(NH_4)SO_4$	3.00 gm
KH_2PO_4	3.00 gm
$MgSO_4.7H_2O$	0.2 gm
$CaCl_2.2H_2O$	0.2 gm
Yeast Extract	1.0 gm

Preparation of Sugar Beet Waste

In order to prepare the waste, it was powdered with the help of a grinder and passed through sieve analysis apparatus.The size (1mm) is select according to [10]

Conversion of Carbohydrate to Sugar

1-Pretreatment of Sugar Beet Waste with Dilute alkali

The following procedure was carried out to delignified the (SBW)[10]:

a-(100)gm of (SBW) was passed through (1mm)mesh and treated with (0.25*M*)of sodium hydroxide (500) ml at 70 °C for 2hr.

b-The delignified pulp was recovered by filtration and thoroughly washed with distilled water until the washing of neutral pH .

c-The washed pulp was stored in moist at 4°C.

d-Drying the pulp in oven at 40° C.

2-Hydrolysis by Sulfuric Acid

The following procedure was followed for the digestion of (SBW) [11].

a-[2*N*] of sulfuric acid was added to pretreatment material by using a solid to liquid ratio of 1:4 .

b-The samples were put in autoclave at (121°C ,15 psig) during(60) min

c-The mixture was cooled after filtration.

d- Neutralization with CaO powder.

e-The concentration of soluble sugar was determined by refractometer

f- Evaporated gently by oven at 40°C to concentrate the solution to 25(%).

Analytical Methods

Brix Value

Brix value (total soluble solid) of (SBW) extract was measured by placing a drop of solution on the refractometers glass .The apparatus was previously standardized with distilled water. The temperature was fixed at 20°C [12].

Measurement of Ethanol Concentration

The ethanol concentration was measured by method of analysis of the American Society of Brewing Chemists in yeast culture dependence on specific gravity measurement by using pycnometer [12].

Measurement of The Population

The number of suspended cells of yeast was calculated by hemocytometer, dilution with sterilized physiological solution (0.9%NaCl) was required to obtain the number of cells per ml for free cell process and immobilized cell process [13].

Preparation of Inoculum

a-Dispress (5gm) of baking yeast in one-liter of physiological solution (0.9%NaCl) at 25°C.

b-Shake the yeast suspension in flask by hand vigorously.

c-Calculate the number of yeast cells in ml.The inoculum a mount was calculated as $6.8*10^6$ cells /ml .

Immobilized Yeast Preparation

Immobilization of Yeast in Activated Charcoal by Covalent Binding

The following procedure was used to prepare immobilized yeast.

a-Various amounts of activated charcoal were taken (2.5,5.8,11,16,20)gm and added to (100)ml conical flask, then sterilized in an oven at a temperature of 160°C for 90min.

b- (10)ml of the yeast slurry with cell suspension ($6.8*10^6$) cell/ml was mixed with activated charcoal for 24 hr at room temperature to ensure complete mixing [14].

c-The charcoal was collected by filtration, washed with (100)ml of cooled distilled water and left to dry [8].

d-The immobilized yeast was stored at (4-8)°C until required to work [13].

Immobilization of the Yeast in Calcium Alginate by Entrapment

The following procedure was used to prepare immobilized yeast [13]

a- 4gm sodium alginate were dissolved in (100)ml physiological solution (0.9% NaCl).The dissolving process of sodium alginate in water was slow so that it required speeding up by warming the solution gently. The solution was then autoclaved at 121°C for 15min under 15 psig .

b-Tewenty conical flasks of (500) ml were provided with (300) ml calcium chloride ($CaCl_2.6H2O$) solution in concentration (0.15*M*) and then autoclaved at 121°C for 15min under 15 psig.

c-(10)ml of spore suspension were added to the solution prepared in step (a) and dissolved with volumes of sodium alginate solution (1.15,3.768,6.377,8.986,11.515) ml which were equivalent to weights to (2.5,5.8,11,16,20)gm and the mixture was stirred to ensure complete dissolution .

d-The spore /Na alginate solution was then added dropwise (with syringe) with stirring to a (0.15 *M*) $CaCl_2$ solution. The gel beads formed were left in solution for 1 hr before being filtered off. The beads were then washed with (100)ml distilled sterilized water (0.9%NaCl)for 20min to allow the diffusion of excess calcium .

Results and Discussion

Effect of pH

The pH has a significant influence on fermentation due as much to its important controlling bacterial contamination as to it is effect on yeast growth, fermentation and by product formation, therefore maintenance of pH is of paramount importance in fermentation processes, the efficiency of the yeast strain was evaluated in the pH rang (3.85-5.3) above pH = 5.3 due to decrease with increasing pH .The experimental results are show in figures 2&3. Figure 2 shows the influence of pH on ethanol concentration the various concentration of glucose (2.5-25%(wt/v) in the presence of 20%(wt/v) activated charcoal .it is obtained that ethanol concentration increases with increasing pH till it reach to 5.3, at this point the value of ethanol concentration is equal to 8.85%(wt/v) at 13.75 %(wt/v) glucose concentration.Then it decreases with increasing pH where it reach to 7, at this point value of ethanol concentration is equal to 7.301%(wt/v).Figure 3 shows the influence of pH on ethanol concentration the various concentration of glucose (2.5-25%(wt/v) in the presence of 20%(wt/v) calcium alginate. It is observed that ethanol concentration was increases with increasing pH .The maximum ethanol concentration can be obtained is 9.22%(wt/v) at pH 5.18 and 13.75%(wt/v) glucose concentration , then it decreased to 8.332%(wt/v) at pH 7.

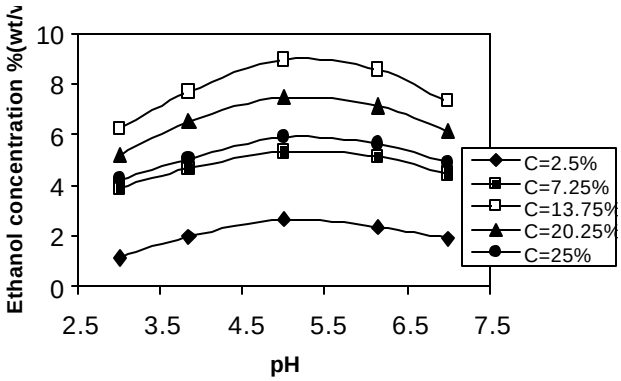


Figure (2) Effect of pH on ethanol concentration at different glucose concentrations in the presence of 20%(wt/v) charcoal

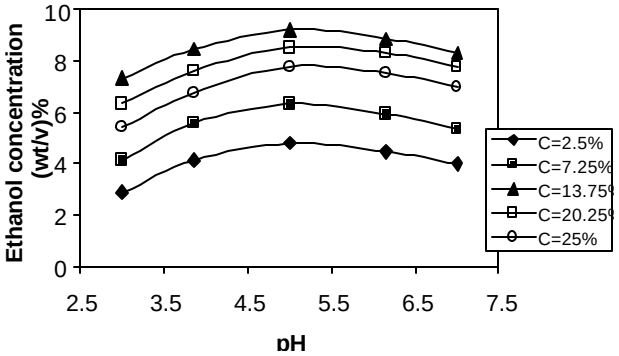


Figure (3) effect of pH on ethanol concentration at various glucose concentrations in the presence20% calcium alginate

Effect of glucose concentration

The results shown in figures4& 5. These figures show the influence of glucose concentration in the range (2.5-25%(wt/v)) on the ethanol concentration at various values of immobilized agent and Optimum pH . The results obtained indicated that the ethanol concentration increases with increasing substrate concentration till it reaches 16.14%(wt/v). At this concentration the ethanol concentration is equal 8.812%(wt/v) at pH 5.3 and 20%(wt/v) activated charcoal, then it reduced to 5.876%(wt/v) at 25%(wt/v) glucose concentration and 20%(wt/v) activated charcoal. In the presence of the optimum value of pH 5.18 the ethanol concentration increased with increasing glucose concentration at different values of calcium alginate concentration in the range (2-20%(wt/v)) shown in figure (5). (4.453 %(wt/v)) was obtained at 2.5%(wt/v) glucose concentration and 20%(wt/v) calcium alginate then it increased with increasing glucose concentration till it reached 16.442%(wt/v), at this concentration the ethanol concentration was equal to (9.3176%(wt/v)) in the presence of 20% calcium alginate and pH 5.18, then ethanol decreased with increasing glucose concentration where it reached 25% (wt/v). At this point the ethanol concentration was equal to (7.112%(wt/v)) at 20%(wt/v) calcium alginate and pH 5.18.

The above results show that the optimum concentration of glucose for ethanol production is (16.14 ,16.442%(wt/v))for activated charcoal and calcium alginate respectively then the above of these concentrations due to decrease of production of ethanol .

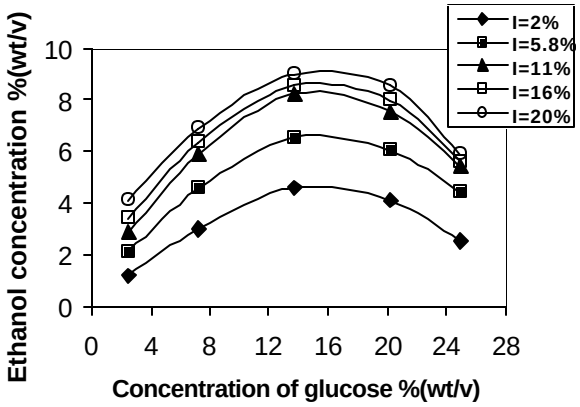


Figure (4) Effect of glucose concentration on ethanol concentration at different value of activated charcoal concentration in the presence of pH 5.3

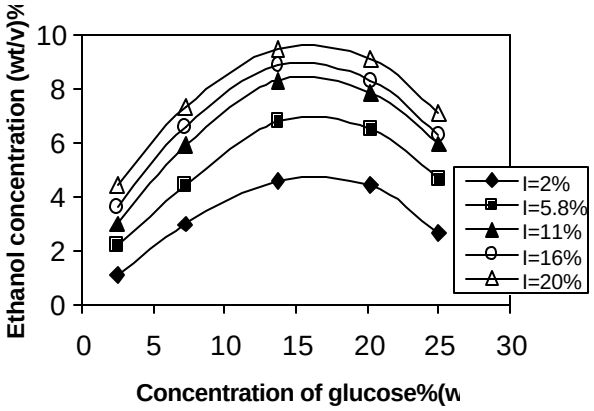
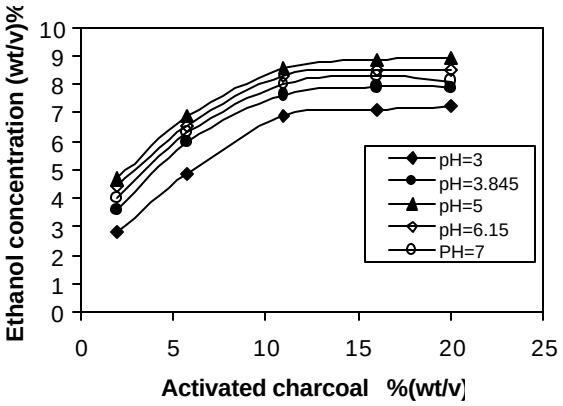


Figure (5) Effect of different glucose concentration and different value of calcium alginate concentration on ethanol concentration at pH 5.18

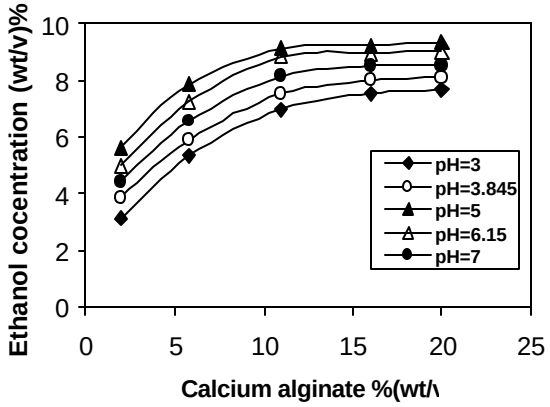
Effect of immobilized agent

Figures 6 & 7 clarify the effect of immobilized agent concentration (2-20%(wt/v)) on ethanol concentration at various values of pH and optimum concentration of glucose. Figure 6 shows how the activated charcoal concentration affects on the ethanol concentration at different pH . It was indicated that the ethanol concentration increases with increasing activated charcoal, where it rises from 4.684%(wt/v)at 2%(wt/v) activated charcoal, pH 5 and glucose concentration 16.14%(wt/v) to 8.8992%(wt/v) at 20%(wt/v) activated charcoal, pH 5 and glucose concentration 16.14%(wt/v) .The influence of different concentrations of calcium alginate at different value of pH at optimum value of glucose concentration is shown in figure 7.



Figure(6) Effect of activated charcoal at various values of pH on ethanol concentration and 16.14%(wt/v) glucose Concentration

It is obtained that ethanol concentration was increased from 5.423%(wt/v)at 2%(wt/v) calcium alginate ,pH 5 and 16.442%(wt/v) glucose concentration to 9.223%(wt/v) at 20%(wt/v) and same pH 5. From the above results it might suggested that the ethanol concentration increases with increasing immobilized agent concentration.



Figure(7) Effect of calcium alginate on ethanol concentration at different values of pH and 16.442%(wt/v) glucose concentration

Effect of time

In fermentation reaction, Inoclum time is very important in obtaining maximum ethanol production with minimum time. Figure 8 shows the effect of time on ethanol concentration at 13.75%(wt/v) glucose concentration, pH 5 and 11%(wt/v) immobilized agent concentration. It was found that the ethanol increases with increasing time, from 7.52%(wt/v) after 1 day to (8.85%(wt/v)) and (9.11%(wt/v)) after 4 days and remains relatively constant after 5 days on for activated charcoal and calcium alginate respectively.

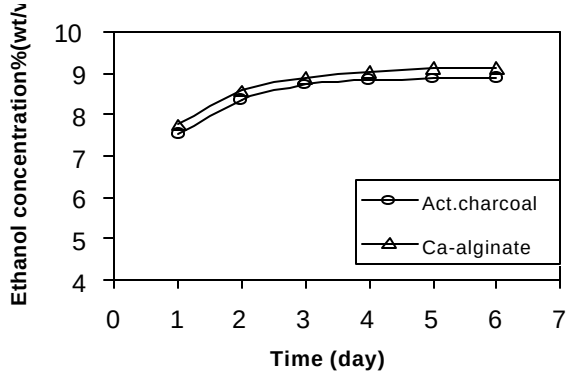


Figure (8) Effect of time on ethanol concentration at 13.75% glucose , pH 5and 11% immobilized agent

Ghanim (1992) found that the ethanol concentration remains relatively constant after four days from glucose by using activated charcoal to immobilized S.cerevisiae [14]. The difference in results may be due to inoculum amount. It is a very important factor.After the particular cell density is reached to growth phase slowly and the life cycle of the yeast deviates from the growth path and produces ethanol. If the cell density is less, more time will be taken for the production of ethanol by fermentation reaction [15].

Effect of shaking

Figure 9 shows the influence of glucose concentration on ethanol concentration at constant operating condition 30°C, pH 5, and11% immobilized agent concentration, were cultivated under aerobic conditions in shaker incubator. It was obtained that ethanol concentration increases with increasing glucose from (4.06,4.28%(wt/v)) at 2.5%(wt/v) glucose concentration to (9.11,9.35%(wt/v)) at 13.75%(wt/v) glucose concentration, then it reduced to (7.11,7.32%(wt/v)) at 25%(wt/v) by using activated charcoal and calcium alginate respectively. In comparison in still culture it was found that increase of ethanol concentration at the same operating condition were cultivated in shaker incubator because this process do under aerobic condition .

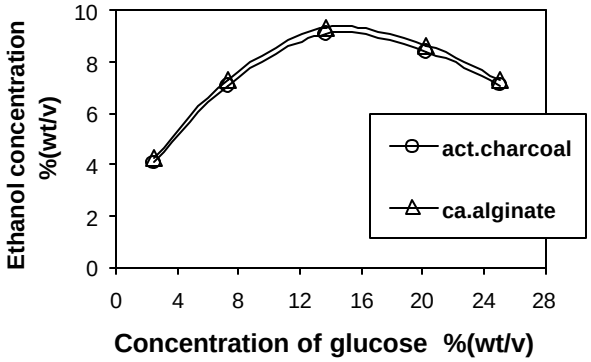


Figure (9) Effect of glucose concentration on ethanol at constant pH 5,30°C ,11% immobilized agent in shaker incubator.

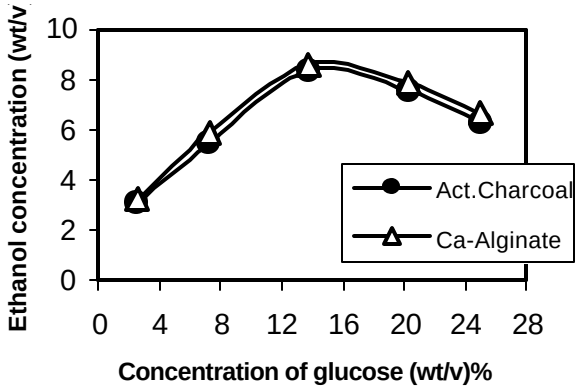


Figure (10) Effect of glucose concentration on ethanol at constant pH 5,30°C ,11% immobilized agent in (without shaking).

Empirical Correlation

The experimental results of ethanol concentration production as a function of pH, glucose concentration and immobilized concentration by second order polynomial are

Y=B0+B1*X1+B2*X2+B3*X3+B4*X1^2+B5*X2^2+B6*X3^2+B7*X1 *X2+B8*X1*X3+B9*X2*X3

where :

X1= pH

X2=glucose concentration

X3=immobilized concentration

Conclusions

The experimental study of the ethanol production from sugar beet waste extract by immobilized cell fermentation has given some basic information. In general, the following conclusions are drawn from the present study:

1-The highest amount of reducing sugar can be obtained by pre-treatment of (SBW) with NaOH that increases the solubility of sugar, and also hydrolysis by H2SO4 (2N) at 121°C, 15 psig for 60 min produce 10brix of sugars (mainly glucose) .

2-Increasing glucose due to the increase of ethanol concentration till it reaches the optimum value and above the optimum value decreases the ethanol concentration The optimum concentration of glucose at maximum ethanol concentration for each immobilized cell (activated charcoal, calcium alginate) are (16.14, 16.442 %(wt/v)) respectively. And the optimum pH of ethanol concentration for immobilized cell (activated charcoal ,calcium alginate) were between (5.18-5.3).

3-Ethanol concentration increase with increasing immobilized agent concentration. The optimum immobilized agent for ethanol concentration for two types is equal to 20%(wt/v). And Calcium alginate was the best immobilized agent compared to activated charcoal with respect to ethanol production.

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