# **Ethanol Bioproduction in Three-Phase Fluidized Bioreactors**

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## Abstract

In the last decade bioalcohol has become more and more important as an alternative energy source and chemical feed stock. Bioethanol production has been proposed as a gasoline enhancer to reduce greenhouse gases, gasoline imports, and to boost the economy.

Circulating fluidized beds (CFB) have been used in a variety of industrial processes due to their distinct advantages of uniform temperature distribution, high gas-to- particle mass and heat transfer rates and flexible operation.

The present study deals with the experimental analysis of the circulating fluidized bed reactor, which is applied to the fermentation of glucose to ethanol. The study takes into consideration the presence of three different phases; yeast (solid) which is continuously fluidized by the liquid stream (glucose solution), and the gas bubbles which greatly enhance mixing and the wake phase which follows tracks of the gas bubbles. The reactor performance is analyzed as a function of major operating conditions, the yeast mass in the reactor (30-150gm/l), the concentration of glucose in feed (10-150gm/l), reaction temperature (15,25,30,36,37, and 40°C), and velocities of gas and liquid feeds (0.01-0.1m/s). The results indicate that high glucose conversions can be obtained at high gas velocities, low liquid velocities, high yeast concentration, and an optimum operating temperature of  $36^{\circ}$ C.

**Keywords:** Bioethanol production; Three-phase fluidization; Bioreactors; Biofuels.

الخلاصة

تضمن البحث دراسة انتاج الايثانول بايولوجيا في مفاعل ثلاثي الأطوار وذو الطبقة المسيلة الدوارة وذلك بواسطة الخميرة النشطة (الطور الصلب) المسيلة باستمرار بواسطة الطور السائل المتمثل بمحلول السكر (الكلوكوز) وبوجود الهواء (الطور الغازي) المحفز القوي على عملية الخلط الجيد0 اجريت التجارب في منظومة تتكون من مفاعل مصنع من الزجاج وبابعاد (قطر 20 سم و ارتفاع 108 سم). تم تقييم اداء المفاعل من خلال دراسة عدة متغيرات شملت كل من تركيز السكر (الكلوكوز) وتركيز الخميرة المستخدمة في المفاعل وسرعة الهواء بالاضافة اللي درجة حرارة التفاعل. حيث اجري تفاعل المستخدمة في المفاعل وسرعة الهواء بالاضافة اللي درجة حرارة من خلال الدراسة تم الاستنتاج ان درجة حرارة مختلفة ( 15، 25، 30، 36، 37 و40<sup>°</sup>م) ومن خلال الدراسة تم الاستنتاج ان درجة حرارة 36 م تعتبر هي درجة الحرارة المثلى لنفاعل التخصر. من خلال الدراسة تم الاستنتاج ان درجة حرارة 16 م تعتبر هي درجة الحرارة المثلى النفاعي التخصر. عالم المواد البحث و التحليل العملي للنتائج تبين انه يمكن الحصول على تحول جيد السكر ومعدل نمو متزايد لخلايا للخميرة باستخدام نسبة مرتفعة من تركيز الخميرة الى تركيز الكلوكوز ومن خلال تدفق مان خلال المواد تم المسلم المنكورة من تعام التخاص معدل نامو من خلال الدراسة تم الاستنتاج العملي النتائية تبين انه يمكن الحصول على تحول جيد السكر ومعدل نمو متزايد لخلايا للخميرة باستخدام نسبة مرتفعة من تركيز الخميرة الى تركيز الكلوكوز ومن خلال تدفق

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## 1. Introduction

In the current time, the importance of alternative fuel has become even more necessary not only due to the continuous depletion of limited fossil fuel stock but also for the safe and better environment, with an inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of environmental friendly biofuels and energies [1, 2].

The catalytic technology plays major role in the different processing stages in a biorfinery for the production of liquid as well as gaseous biofuels. There are number of challenges to find suitable catalytic technology to be used in a typical biorefinery. These challenges include (1) economic barriers, (2) catalyst that facilitate highly selective conversion of substrate to desired products and (3) the issue related to design, operation and control of catalytic reactor [3].

Bioethanol is regarded as one of the most important renewable fuels. Bioethanol is clean, efficient burning, octane enhancer, and highly combustible renewable fuel. It is simply ethanol ( $C_2H_5OH$ ) derived from agricultural sources and it is predominantly produced through the fermentation of starch or sugar found in products such as grain and sugarcane. This process is described by the global reaction [4, 5].

$$C_6H_{12}O_6 \xrightarrow{yeast} 2C_2H_5OH + 2CO_2$$

There are many types of processes and bioreactors used for the production of bioethanol; batch bioreactor, fixed bed bioreactor and fluidized bioreactor. In recent years, the production of alcohols in three- phase fluidized bioreactors represent a very promising and challenging opportunity to the engineer and scientist.

Circulating fluidized bed (CFB) reactors are intensively used as multiphase contactors and reactors in chemical. petrochemical biochemical and industries. They are applied in various types of processes involving gas-solid contact because of their excellent mixing and transport characteristics. Circulating fluidized bed reactors are widely used in biochemical applications where microorganisms are utilized as solid suspensions in order to manufacture industrially valuable bioproducts [4, 5, 6].

In CFB bioreactor the gas and liquid feeds are introduced in a cocurrent fashion at the lower part of the column. Yeast particles can be added to the reactor through feeding parts at the top part of the reactor. Gas is present either because of the aeration requirements of the cells or as a product of their metabolism. Usually, fluidization is obtained either by external liquid recirculation or by the gas loaded or produced in the reactor. These main characteristic make the use of threephase fluidized configurations extremely suitable for conducting biochemical reactions [7, 8].

Many fermentation processes involve microorganism attached to solid particles. In these systems, investigations aim to maintain high cell concentration at an optimal reaction conditions. Cell growth is an autocatalytic reaction which is characterized by variables such as the reaction rate and the yield of product from substrate. A functional relationship between the growth rate and an essential compound's concentration was proposed

by Michaelis-Menten in 1942. An essential feature of Michaelis - Menten kinetics is that the catalyst becomes saturated at high substrate concentrations. Therefore, the reaction rate approaches a constant value independent of substrate concentration; in this concentration range, the reaction is essentially zero-order with respect to substrate. On the other hand, at low substrate concentration Michaelis-Menten reactions are essentially first order with respect to substrate [4,8].

Fluidized bed bioreactors have received sustained attention that makes them an alternative in bioethanol attractive production. However, their full implementation at the production scale has been quite limited up to now. A number of important issues for the characterization, design, and operation of fluidized bed bioreactors have been investigated by many authors. Quintero and co-workers studied the ethanol production process from sugarcane and corn. They found that sugarcane ethanol process was determined as the best choice for ethanol production than the starchy crops of corn [4]. In addition, authors studied ethanolic many fermentation by different types of microorganisms. They studied their role in the catalytic process for the production of biofuels [9,10,11].

This paper focuses on different parameters characterizing the operation of circulating fluidized bed reactor. provide which in turn better understanding to the critical factors that control the successful operation of converting the renewable feed stocks into the desired biofuel

# 2. EXPERIMENTAL WORK 2.1 Materials

In present investigation, various types of pure chemical compounds are used such as, glucose (99.9%), MgCl<sub>2</sub> (99.8%), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (98%) and NH<sub>4</sub>Cl (99.6%) were obtained from Fluka AG Company. The organism used in this study was commercial baker's yeast (Type DZ), obtained from Deutche Hefewerke DHW.

# 2.2 Bioreactor System

Figure (1) shows a schematic diagram of the circulating fluidized bed (CFB) reactor. The reactor is constructed from Plexiglas cylinder of 20 cm diameter and 108 cm height. The reactor is fitted with four sampling points, which are placed at equal intervals between the bottom and the top part of the reactor. Feed streams into the reactor are air and glucose solution. The air stream is uniformly bubbled in the liquid phase via perforated ceramic tube. On the other hand, the feed glucose solution is distributed through a coarse bed of glass beads placed in the lower section of the reactor. Phase separation, i.e., solid, liquid, and gas is achieved by fitting the top part of the reactor with a cylindrical tube of large diameter. As a result, solids settle back into the reactor, while gas bubbles coalesce and escape through the reactor top opening. The rising liquid phase exits the reactor through a side part.

The feed sugar solution is kept in a storage tank which is fitted with an immersion heater to maintain constant temperature throughout the experiment. Also, another immersion heater is positioned in the reactor top opening, to ensure temperature uniformity. During experiments both heaters are set to the same temperature reading. Fluidization of the reactor content is achieved by the use of a variable speed pump.

## 2.3 Experimental Procedure

The operation of circulating fluidized bed reactor required preparation of the glucose feed solution of specified concentration. The solution is prepared and mixed in the feed tank where part of it is pumped through the system to the fill the reactor. During the initial preparation period, the temperature of both immersion heaters is adjusted and kept constant to reach steady state conditions. This is followed by adjustment of the gas (air) feed flow rate. Table (1) summarizes the operating parameters and conditions that were performed for the bioconversion in the CFB reactor.

On the other hand, the Bakers yeast solution is prepared in a separate flask, where it is dissolved in two liters of water together with all nutrients, i.e., MgCl<sub>2</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and NH<sub>4</sub>Cl, necessary to sustain proper growth of the yeast cells. Experimental measurements start with addition of the yeast solution through the top opening of the reactor. After, 1 minute the feed pump is operated and the purge stream is opened. This delay is necessary to insure proper mixing of the yeast solution in the rector, and to avoid carry over of the yeast cells in the purge steam.

The samples of liquid are collected at constant time intervals from the four sampling points along the reactor. The samples are divided into three portions which are analyzed separately for yeast, sugar, and ethanol concentrations. The yeast concentration is measured using turbidity meter for the raw sample. An Ultra-Violet spectroscopy analysis at wave length of 494 nm, of the filtered solution is used to determine sugar concentration. The measurements of ethanol concentration are necessary to verify conservation of mass within the systems and in turn validity of other measurements. Therefore, the filtered solutions were analyzed using a Shimadzu GC-2014 gas chromatograph coupled with FID, using S.G.E. capillary column of 25 m length and 0.22 mm inner diameter.

# **3. RESULTS AND DISCUSSION**

The following analysis focuses on characterization of the conversion of glucose into ethanol by yeast in a threephase fluidized bed reactor. To accomplish this task several experiments were performed covering a wide range of yeast and glucose concentrations, reaction temperatures, and liquid and gas velocities. **3.1 Effect of Temperature** 

The reaction temperature has a direct effect on the activity of the yeast and in turn the rate of glucose conversion.

Figure (2) and (3) show the overall glucose conversion is strongly affected by the reaction temperature decreasing from 17% at 36°C to 3% at 15°C. An increase in the maximum growth rate occurs as the temperature increases from  $30^{\circ}$  to  $36^{\circ}$ C. This behavior is reversed as the temperature increases from 36° to 40°C. This behavior is attributed to the decease in yeast activity as the reaction temperature is increased. The same observation is simulated by the maximum specific ethanol production obtained from Bauer's experimental work [12]. Figure (3) shows that the peak conversion temperature is 36°C.

This direct effect on the activity of the yeast and in turn the rate of glucose

conversion agrees with the results reported by Bauer [14].

# 3.2 Effect of Yeast to Glucose Ratio

Glucose and yeast concentrations within the reacting mixture are major operating parameters which directly affect rate of ethanol production. To study the effect of the two parameters, the glucose in feed varied between 10 and 150 gm/L, and yeast concentration within the reactor varied between 35 and 150 gm/L. Effects of glucose concentration in feed on glucose conversion, and on ethanol production are summarized in Figures (4), (5), and (6). At the lowest glucose concentration. 10 gm/L, or а glucose/yeast ratio of 0.2, almost complete conversion is realized. On the other hand, a glucose conversion of 48% is reached at an equal glucose and yeast concentrations. Further increase in the glucose concentration results in a much lower conversion, i.e., close to 12%. It is found that at small feed glucose concentration, the product inhibition caused by ethanol produced can be avoided. However, at larger glucose concentration, the inhibition effect should be considered. Therefore, it is recommended for future work, the ethanol produced should be continuously removed from the reaction mixture.

Figure (4) indicates that, the transient time to reach steady state conditions increases with increased glucose concentration, i.e., 10 minutes for 10 gm/L and more than 30 minutes for 150 gm/L.

Figure (6) confirms Monod kinetics characteristics that at high glucose concentration the exit conversion seems not very sensitive to changes in inlet glucose concentrations, i.e., reaction order approaching zero.

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The effect of yeast concentration on exit glucose conversion, and on ethanol production is displayed in Figures (7), (8), and (9). The results show that a high glucose conversion depends strongly on the yeast concentration.

The same observation has also been reported by the work of Quintero [4] and Roca et al. [11]. The drop in yeast concentration along the reactor is caused by two mechanisms. The first involves formation of excessive foaming at the reactor top which results in carry over of the yeast cells with air bubbles leaving the reactor. The second mechanism is due to the carry over of the yeast cells in the exit stream which causes continuous depletion and in turn decreases in the veast concentration. The foaming problem can be solved by the use of antifoaming agent which was not available at the time of experiments. The other problem of yeast carry-over is more complicated and it requires increase of the yeast particle size to ensure proper settling and eliminate carryover.

# 3.3 Effect of Liquid and Gas Velocities

Effects of varying the liquid and gas velocities are studied to assess both dispersion and residence time effects. The data are shown in Figure (10), where the air velocity increased from 0.01 to 0.1 m/s at liquid velocity 0.01, and 0.015 m/s. Increasing the gas velocity reduces the liquid Peclet number, consequently, the dispersion coefficient of glucose and ethanol in the liquid phase are both increased resulting in higher conversions, as shown in Figure (10) for glucose concentrations of 100 and 150 gm/L. In addition, an increase in gas velocity would lead to an increase in axial movements of gas

bubbles and wakes which are the main cause of axial mixing in the direction of flow in three-phase fluidized bed. In this case, the net effect is the high dispersion coefficient which leads to a better mixing and uniform conversion through the reactor, as shown in Figure (11). On the other hand, effects of liquid velocity are glucose conversion on less pronounced. This is shown in Figure (10), where very small changes in glucose conversion are resulted as the liquid velocity is increased from 0.01 to 0.015 m/s at both glucose concentrations. The slight drop in exit conversion is probably due to the drop in liquid residence time. This conclusion is in agreement with the conclusions that submitted by the work of Juray [13], and Kim [15].

## 4. Conclusions

This study presented analysis of a three phase fluidized bed reactor and its experimental verification using bioconversion of glucose into ethanol by yeast. The study predicts axial profiles of glucose, and ethanol, as a function of time. The analysis covered a range of major operating parameters, including yeast concentration in the reactor, operating temperature, and velocities of liquid and gas streams. The results show that high glucose conversion achieved at high ratios of yeast to glucose concentration in the reaction mixture, large gas velocities and at optimum temperature of 36°C. The present work is a very useful tool to further study the ethanol-glucose system as well as other three-phase fluidized bioreactors. The research reported herein can be used to predict a time dependent and onedimensional model to analyze the performance of three-phase fluidized

bioreactors. Its prediction can be used to select better operating conditions, optimum parameters, and to aid in the design of bioreactors.

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Table (1) The summary of the operating variables and conditions			
in CFB reactor.			

	Variable	Range	J <b>nit</b>
1	Sugar Feed Concentration	10, 50, 100, and 150	(gm/L)
2	Yeast Concentration	35, 50, 90, and 150	(gm/L)
3	Air Flow rate	19	(L/min)
	Air Velocity	0.01	(m/s)
4	Liquid Flow Rate	28.5	(L/min)
	Liquid Velocity (U <sub>1</sub> )	0.015	(m/s)
5	Reaction Temperature	15, 25, 30, 36, 37, and 40	°C



Fig. (1) Schematic diagram of the experimental apparatus.



Fig. (2): Variation in glucose conversion at reactor exit as a function of time and reactor temperature



Fig. (3): Effect of reactor temperature on exit glucose conversion.



Fig (4) Variation in glucose conversion at reactor exit as a function of time and its feed concentration.



Fig.(5) Variation in ethanol production at reactor exit as a function of time and glucose concentration in feed stream



Fig. (6): Variation in glucose conversion and ethanol production at reactor exit against its concentration in feed stream.



Fig. (7): Variation in glucose conversion as a function of time and yeast concentration.





# Fig. (8)Variation in ethanol production as a function of time and yeast concentration



Fig. (9): Effect of yeast concentration on exit glucose conversion and ethanol production.

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Fig. (10): Effect of gas and liquid velocities on exit glucose conversion



Fig. (11): Axial profiles of glucose conversion as a function of time.