

## Petroleum Single Cell Protein Production

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### Abstract

This study focuses on producing single cell protein from petroleum fraction (ethanol, kerosene, gas oil) by growing two types of microorganisms (*Candida sp.* and *Bacillus subtilis*) on these materials as energy and carbon source. These two types of microorganisms are isolated from contaminated soils with petroleum derivatives brought from AL-Dorah and Beji refineries, fuel stations, and garages. At the first stage the microorganisms are cultivated on different media (blood and nutrient agar) and on mineral salt medium (MSM) to identify the genus of each type. After this, the microorganisms are grown on different concentrations of ethanol, kerosene, gas oil (0.5, 1.0, 2.0, 3.0, 4.0 (v/v)%) at 7.4 pH and 32°C temperature. In the second stage the microorganisms are growing in still liquid culture at the same conditions. In this stage the yield percent, number of cells per milliliter, and biomass weight are calculated for either type of microorganisms. Composite rotatable Box–Wilson experimental design is adopted to design the experimental work. Statistically, the response functions (i.e. yield percent of ethanol, kerosene, gas oil) are related to the most effective variables on single cell protein (SCP) production. The temperature is fixed at 32°C at this stage also, because the range of temperature growth of these microorganisms is narrow (30 to 34°C), where there is no significant effect at this range of temperature. Concentration and pH are ranged between 0.5 to 4.0% and 7.0 to 9.0 respectively. Optimum conditions are determined and found equal to 7.36 for pH and 1.31% for concentration for ethanol, 7.42 and 4.0% for kerosene, and 7.51 and 4.0% for gas oil. The percentage of crude protein was evaluated according to Kjeldhal method. The maximum protein content was 61.25% which was closed to the results obtained by previous studies. The bioassay was carried out using chicken embryos, where chicken eggs were used for this purpose. The result of bioassay was 83.5% of successful eggs, which is acceptable with the percent given by Kohen Lee. The last stage is designing a batch bioreactor and calculating all process parameters depending on the results of experimental work in previous stages. In general, the characteristics of (SCP) production using two types of microorganisms are agreed well with general characterization of (SCP) production that accommodates the general concepts of (SCP) production.

**Keywords:** SCP Production, Single Cell Protein

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## انتاج بروتين احادي الخلية من المشتقات النفطية

### الخلاصة

تتركز هذه الدراسة على إنتاج البروتين الأحادي الخلية من المشتقات النفطية (الكحول الأيثلي , النفط الأبيض , وزيت الغاز ) وذلك بتتبع نوعين من الأحياء المجهرية ( *Candida sp.*, *Bacillus Subtilis* ) على هذه المواد حيث تعتبر كمصدر للطاقة و الكربون لنمو تلك الأحياء. عزل هذين النوعين من الأحياء المجهرية من ترب ملوثة بالمخلفات النفطية حيث جلبت من مصفى الدورة ومصفى بيجي ومحطات الوقود ومراب السيارات . في المرحلة الأولى زرعنا الأحياء المجهرية على أوساط نمو مختلفة ( MSM Nutrient agar , Blood agar ) وذلك للتعرف على جنس ونوع كل من الكائنات المجهرية , بعد ذلك نميت الأحياء المجهرية بتراكيز مختلفة من الكحول الأيثلي , النفط الأبيض , وزيت الغاز ( 0.5 , 1.0 , 2.0 , 3.0 , 4.0 (v/v)% ) عند pH (7.4) ودرجة حرارة (32°C) . في المرحلة الثانية نميت الأحياء المجهرية في مزرعة رابدة سائلة وبنفس الظروف السابقة في هذه المرحلة حسب الإنتاجية وعدد الخلايا بالملييلتر و وزن الكتلة الحيوية لكلا النوعين . في المرحلة الثالثة من الجزء العملي طبق التصميم التجريبي بطريقة (Box -Wilson) لتصميم العمل التجريبي . وتم ربط دالة الاستجابة (الإنتاجية) إحصائياً مع أهم العوامل المؤثرة على إنتاج البروتين الأحادي الخلية وهي ( الأس الهيدروجيني pH و تركيز المادة الخاضعة (substrate) ) باستعمال نظام الدرجة الثانية. طبقت المديات الآتية للمتغيرات : ( 7.0- 9.0 ) للـ pH و ( 0.5-4.0 % ) للتركيز , أما بالنسبة لدرجة الحرارة فليس لها تأثير يذكر وذلك لضيق المدى الذي تنمو فيه هذين النوعين من الكائنات المجهرية حيث يتراوح ( 30-34°C ) , تم تعيين الظروف المثلى , وكانت (1.31%) للتركيز و (7.36) للأس الهيدروجيني pH بالنسبة للكحول الأيثلي (ethanol) , أما بالنسبة للنفط الأبيض (kerosene) فكانت (4.0%) للتركيز و (7.42) للأس الهيدروجيني pH أما بالنسبة لزيت الغاز فكانت (7.51) للأس الهيدروجيني و (4.0%) للتركيز . أما نسبة البروتين الخام فقد حسبنا وفقاً لطريقة العالم ( **Kohen Lee** ) أما أعلى قيمة للبروتين الخام حيث كانت (61.25%) وهي مقارنة للنسبة التي توصل إليها الباحثين السابقين . أما الاختبار البايولوجي للمنتج فقد تم باستخدام اجنة الدجاج ( بيض الدجاج ) وقد تم الحصول على نسبة نجاح (83.5%) وهي أعلى من النسبة التي حددها ( **Kohen Lee** ). أما المرحلة الأخيرة فتمثلت بتصميم مخمر من نوع الوجبة وتم حساب جميع المتغيرات المتعلقة بهذه العملية . بصورة عامة اتفقت خصائص البروتين المنتج مع الخصائص العامة المحددة لإنتاج هذه المواد .

### Introduction

#### Single Cell Protein (SCP)

The term of single cell protein refers to dead, dry cells of microorganisms such as yeast, bacteria, fungi, and algae which grow on different carbon sources. The name single cell protein was used for the first time by the professor ( **Carol Wilson** ) to give a better image than **microbial protein**.<sup>[12]</sup> The microorganisms which used for this purpose contain nutrient matters like carbohydrates, fats, vitamins, and minerals. <sup>[14]</sup>The high growth rates of microorganisms

,the high efficiency of their substrate conversion , and inexpensive raw materials from the commercial basis for production SCP. Other advantages of SCP over conventional protein high yields, and possibility of continuous harvest . There is a variety of carbon sources available to produce SCP as CO<sub>2</sub> ,carbohydrates,hydrocarbons,lipids.<sup>[15]</sup> Many process have been developed to produce microorganisms able to use organic materials as a source of carbon and energy and to convert inorganic nitrogen into high feed value proteins called **Single Cell Protein**

(SCP). These can be used in human foodstuffs or animal feed to replace traditional plant or animal sources. The various microorganisms used for biomass production and the various metabolic pathways involved in substrate catabolism are described here. Physiological aspects, growth parameters, energy, and nutritional requirement and influence of physicochemical parameters are discussed. The different types of microbial culture and examples of process are described.<sup>[16]</sup> Petroprotein or petroleum protein is concentrated that is produced by growing specific microorganisms in petroleum fractions, derivatives of hydrocarbons (alcohol), or natural gas in addition to other chemicals essentials. Given adequate engineering and biochemical conditions, these microorganisms multiply at phenomenal rate. The end product is concentrated which is further separated, purified and then prepared for consumption its protein contains from sixty to eighty percent and has been successfully used as animal feed.

#### Material and Method

The experimental part carried out on production of (SCP) by using microbial fermentation described according to Box – Wilson experimental design in order to find the optimum operating conditions that correspond to maximum yield of (SCP). In general the following steps were followed :

1. Isolation of microorganisms from petroleum soil.
2. Growing the microorganisms on plate of (Agar medium).
3. Classification of microorganisms.
4. Testing the isolated microorganisms by growing them in still culture on different substrates (**Kerosene**, **Ethanol**, **Gas oil**) with different concentration at pH (7.4).
5. Study the influence of most dominate parameters (**pH** and **Concentration**) by growing the microorganisms in liquid culture with shaking, according to Box – Wilson design.

6. Estimate the crude protein percent according to Kjeldhal method.
7. Bioassay of the protein on chicken embryos.
8. Design pilot batch bioreactor and calculate all batch process parameters depending on experimental results.

#### Preparation Media of Growth

For most agar-based media the powdered medium was mixed with water and steamed to dissolve the agar, the whole was then sterilized in an (Autoclave) at (121°C) for 15 min and (15 psi) and subsequently allowed to cool to about (40°C), a temperature at which the agar remains molten. To prepare a plate some (15 – 20) ml of molten agar was poured into sterile Petri-dish which was left undisturbed until the agar solidified.<sup>[11]</sup>

#### Preparation Mineral Salt Medium (MSM)

*Sungpetch Acharaporn et al*, (2002).<sup>[2]</sup>

suggested a suitable salt medium for microorganisms that grow on hydrocarbons, composite of the following materials per one liter of distilled water (K<sub>2</sub>HPO<sub>4</sub> 1.8g, KH<sub>2</sub>PO<sub>4</sub> 1.2g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>2</sub> 4.0g, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.2g, Agar-Agar 2.0g, Yeast- Extract 1.0g).

#### Preparation of Inoculum

The following steps were carried out to prepare inoculum :

1. A laboratory equipment were sterilized by (Autoclave) at (121°C) and (15psi) for (15 min).
2. Samples of contaminated soil were collected from the surface to about (10 – 20 cm) deep, where obtained from several locations (**Begi refinery**, **Al - dora refinery**, **fuel stations**, and **guarages**) in iraq.
3. Samples were stored in clean plastic bags packed with cooling less than 5°C.
4. Five grams of contaminated soil were taken, then put in (100 ml) of sterilized distilled water
5. Soil suspension was shaken vigorously for (5.0 min) using a shaker, figure (3.3) to about (200 rpm) then, let it for settling.

6. (100 ml ) of ( MSM) solution was prepared then , sterilized by autoclave to molt agar then, cool it to about (40 °C ).
7. Molten (MSM) was spilled into three (20 ml ) plate then , put ( 1.5 ml) of **Kerosene**, **Gas oil** , and **Ethanol** to each plate respectively with ( 1 ml ) of soil suspension.
8. (1ml) of soil suspension was put , then spread it on the plate surface .
9. Incubate the plates at (32°C) for( 48 hr ) ,where colonies present at this step have different external morphology .

#### Still Batch Culture

The microorganisms were cultivated in a medium containing ( **0.5** , **1** , **2** , **3** , **4** ( **vol/vol** ) % ) of gas oil , kerosene , and ethanol as a carbon source , where culture grown in ( 250 ml ) Erlenmeyer flask with( 100 ml ) of nutrient medium and incubated at (32 °C) .

#### Batch Culture with Shaking

The microorganisms were cultivated in a medium containing ( **0.5** , **1** , **2** , **3** , **4** ( **vol/vol** ) % ) of gas oil , kerosene , and ethanol with different values of pH adapted according to the ( Box – Wilson ) , these values are ( **7** , **8.3** , **8.7** , **9** ) .At Constant temperature 32 °C was used .The incubation with shaking was at (200rpm).

#### Identification and Classification of Microorganisms

Sample (1) was cultivated on different media (MSM , blood agar , nutrient agar). After 24-48 hour of incubation in the incubator at 37°C , few colonies from the surface of such media were taken by the platinum needle and streak Depending on Bergge 's manual [3]The sample was isolated and identified ( *Bacillus Subtilis* ): is gram – positive . rods (bacillus) , occurring in chains saprophytic organisms prevalent in soil and water . Aerobic or Facultating , spore forming Bacillus , not pathogenic , grow well on blood agar , producing colonies , usually large , flat with ground glass appearance ,

catalase positive . figures (10) and (11) show the growth *Bacillus Subtilis* .

#### The Sample No. Two

The sample was isolated and identified as : (*Candida sp .* ) , see figure (12) and (13). (*Candida sp .* ) : a genus of yeast – like fungi characterized by producing yeast *mycelia* , *pseudomycelia* and *blastospores* . In sample number two , the germ tube test is done . This test is done to differentiate between(pathogenic and non pathogenic candida) , where the result was ( **negative germ – tube** ) .

#### Protein Percent Determination

The total nitrogen was determined by (semi **Kjeldahl Method**) according to AOAC. The procedures of Kjeldahl Method is as follows:

##### Digestion:

1. Prepare 1 ml of sample (containing 3 mg of the matter).
2. Put on it 1 ml of sulfuric acid of concentration 98%, plus 1 ml of copper sulfate 4% , plus 0.8 gm of potassium sulfate .
3. Heat slowly ,then boil vigorously through 20 min ., the color becomes clear , yellow or green when the digestion complete.
4. Reduce and cut off the heat.
5. If the color is not clear in 20 min., reduce the heat, carefully, then add 2-3 drops of hydrogen peroxide 30% And then continue heating for 5-10 min.
6. Let it to cool.
7. Add 7 ml of 0.01N of hydrochloric acid .
6. Add trace of methyl red indicator solution.

##### Distillation:

1. Digest in Kjeldahl flask.
2. Add 7 ml of sodium hydroxide 30% to the mixture through the funnel .
3. Put in the receiving flask 10 ml of boric acid 4%
4. Distill for 3-4 min.

##### Titration

Titrate with standard 0.01N of sodium hydroxide (**NaOH**)

Where 1 ml (0.01N HCl)  $\equiv$  0.14 mg N<sub>2</sub>

$$T.N \% = \frac{ml.HCl \times 0.14}{weight (mg)} \dots(1)$$

$$C.P \% = T.N \% \times 6.25 \dots(2)$$

### Procedure of The Bioassay

The following procedures were adopted according to the principals given by Kohen Lee :

1. Prepare 100 chicken eggs according to the qualifications described above.
2. Use ten eggs in each experiment, half of the eggs were injected and other were not.
3. Inject the eggs with a certain dose (1 gm/l) of protein suspension at the air hall without exposing the eggs to deflect, using special needle ( has a small diameter of about 1 mm).
- 4.Each dose must be obtained from grown yeast on different gas oil concentration (0.5, 1.0,2.0,3.0,and 4.0%).
- 5.Incubate the eggs for 21 days at 35 °C and 20% humidity.
- 6.Calculate the absolute killing percent according to the equation given by **Kohen Lee** 
$$\left[ \left[ \begin{array}{l} \text{Killing} \\ \text{Percent} \end{array} \right] = \frac{B - C}{D - C} \times 100 \right] \dots(3)$$
- 7.Calculate the average killing percent.
- 8.Calculate the success percent

$$\text{Success \%} = 1 - \text{average killing percent} \dots(4)$$

### Results and Discussion

In figure (1) it could be observed that the amount of protein and nitrogen content increases with increasing the gas oil concentration that mean increasing biomass obtained.

Figures (2) and (3) show the relationship between the amount of produced biomass and substrate concentration, of the fermented two types of microorganisms. The growth rate can be represented by counting the number of cells for both types, using the three substrates (i.e. ethanol, kerosene , and gas oil ) with different concentrations as described above As shown in figures (1) and (2) . Generally it could be seen that growth rate , yield , and amount of biomass increases by increasing

the concentration of both kerosene and gas oil , while for ethanol growth rate , yield and biomass decrease by increasing ethanol concentration more than 1.3 % . High values of yield and biomass were obtained when using concentrations between 0.5–1.3 % .

These results are close to the results obtained by **Orlava,(1980)**<sup>[5]</sup> , who suggested that 0.5–1.5% of ethanol gives optimal yields of protein.He also found that when the concentration of ethanol is more than 3% lower yield will be obtained, this fact complies with the results obtained in this investigation .This nearly agreed with the results reported by **Omata,(1970)**<sup>[4]</sup>,who found that the concentration of ethanol is more than 3.2% it will inhibit the cell activity and reduce the velocity of biochemical reaction .

For kerosene and gas oil , it is found that the highest yield obtained when the concentration of these materials is 4% .This means that the concentration of these two materials can be increased to obtain higher yield, but within the limits defined by **Coony,(1972)**<sup>[3]</sup> , who suggested that the most suitable concentration is between 3 – 15% to produce SCP . This percent could reach to 25% as reported by **Killberg,(1970)**<sup>[8]</sup>.The reason of using high concentration of both kerosene and gas oil more than that of ethanol is the variation of ( *n*-Alkanes ) percent in these two materials . This percent varies according to the variation of nature of the crude oil ; therefore it required large amount of kerosene and gas oil to produce the required amount of protein . High percent of biomass yield obtained from gas oil and kerosene 0.886 and 0.943 respectively , where this complied with the range given by Litchfield <sup>[9]</sup> , who obtained 0.88 –1.1 g/g of protein yield from *n*-alkanes .Generally , the increasing in *n*-alkanes percent in petroleum fractions ( kerosene or gas oil ) leads to the increase of the biomass yield because ,the microorganisms are more degradable to these substances than other types found in petroleum fractions.

**Biomass Yield in Batch Culture with Shaking**

In this stage of experimental work , *Candida sp.* was used only , because its consider more economical dute it did not need many separation process ,due to its large size about 5 micron ;therefore its easily to separate in centrifugation or filtration process. Moreover less acceptable to undesired mutations than bacteria .It seems that experimental data obtained from this stage are modeled according to Box-Wilson technique .It is taken in consideration the effect of two variables (i.e. substrate concentration, and pH of culture media) .The results of this stage were shown in table (2).

**Determiration of Second Order Polynomial**

**The Coefficients Calculation**

Using statistica software, the coefficients of the second order polynomial ,equation (5) ,were estimated using a nonlinear regression analysis move method .

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_1^2 + B_4X_2^2 + B_5X_1X_2 \dots \dots \dots ( 5 )$$

Accordingly, second order polynomial equations representing the protein yield for three samples (Ethanol, Kerosene, Gas Oil) in terms of concentration of the substrate and pH of the media of fermentation were written as fellow :

$$Y_1 = 81.2976 - 14.4555X_1 - 1.6956 X_2 - 4.4055 X_1^2 - 3.33302 X_2^2 - 4.4900 X_1 X_2 \dots \dots \dots (6)$$

$$Y_2 = 72.29932 + 11.4618 X_1 - 4.31291X_2 + 2.169015 X_1^2 + 1.443796 X_2^2 + 5.197463 X_1X_2 \dots \dots \dots (7)$$

$$Y_3 = 90.19961 + 10.27490X_1 + 0.492274X_2 - 6.02970X_1^2 - 9.06378X_2^2 + 2.65000X_1X_2 \dots \dots (8)$$

**Effect of pH**

Figures (4),(5),and (6) show the effect of pH value on biomass yield at various concentrations . For ethanol substrate figures show that the yield percent increases until the maximum value reaches (91.4%) after this

value it decreases. Figure (5)shows for kerosene substrate that the yield percent increase until the maximum value reaches (93.2%) then it decreases . Figure (6)shows for gas oil the yield increases until it reaches the maximum value (94.1%) then it decreases.

Obviously a higher yield can be obtained at pH value between 7.0– 7.4, the decreasing after this range due to the cells loss biological activity to do the metabolism process properly, where this fact reported by *Bohlman,(1979)*<sup>[9]</sup>who suggested that the most suitable pH range to produce SCP is 7.0– 7.8 .Also Lain suggested that the most suitable range of pH for yeast which are utilize the hydrocarbones is between 7.0 to 8.3 <sup>[11]</sup> .For ethanol , kerosene and gas oil respectively , the optimum conditions are 1.31% for ethanol , 4.0% for kerosene , and 4.0% for gas oil.

**Effect of Substrate Concentration**

Figures (7),(8),and (9) show the effect of substrate concentration on biomass yield .For ethanol figure (7) show that the protein yield increase with increasing ethanol concentration until reaches the maximum value (91.2%) after this decrease because high concentration of ethanol more than 1.5% will inhibit the biochemical reaction of the cells as described before . For kerosene and gas oil the yield percent of biomass increase with increasing the substrate concentration for both kerosene and gas oil , where it reaches (94.3%) for gas oil and (93.1%) for gas oil , see figures (8) and (9). The optimum values of pH , which were 7.36for ethanol , 7.42 for kerosene , and 7.51 for gas oil .

The above results agree with the results reported by many investigators like *Coony,(1972)* <sup>[3]</sup> , who suggested that the most suitable concentration is between 3 – 15% of normal alkanes to produce SCP. This also agrees with the range given by Litchfield <sup>[9]</sup> , who obtained 0.88 – 1.1 g/g of protein yield from n-alkanes. Orlava <sup>[5]</sup> , also suggested that 0.5–1.5% of ethanol gives optimal yields of protein, where this range

was closed to the range obtained in this investigation.

### Results of Bioassay

Table (1) shows the different results in killing percent of embryos. The difference due to many reasons, either increasing in gas oil concentration in the injected protein matter, or due to reasons which are described later. The increasing in the gas oil concentration in the dose, did not contribute directly in raising the killing percent in embryos. At (0.5%) gas oil, the killing percent was (11.1%) and (1.0%) was (28.6%), while at (2.0%) of gas oil there is no killing percent. This could justify the results of killing percent. There are another reasons might contributed in raising killing percent like fertilization percent of some eggs less than 85%, appendages in structure of some eggs, incubating and injection conditions. To avoid or reduce these problems ten eggs were used in each experiments, five eggs treated and another did not treated. This principal of injection to increase the probability of successful eggs. Generally, its obtained (83.5%) of successful eggs, its considered more than the percent suggested by Kohen Lee (75%). This percentage is closed to the percentage obtained by *Michael .Walsh, (2000)*<sup>[6]</sup>, where they obtained (89.5%) of eggs were successful.

### Conclusions

The following conclusions can be listed from this study:

- To study the characteristics of production (SCP), a mathematical correlation expressing the yield percent is made with two variables that present the most effective parameters (i.e., concentration of the substrate, and pH) which adequately describe the behavior of the process throughout the ranges of the studied variables.
- It is shown that the two variables studied affect on SCP production in following sequence: substrate concentration, and pH.
- It seems that the temperature does not have significant effect on the growth rate for *Candida sp.* because, narrow range of growth temperature (30 – 34 °C).
- It seems that the increasing the pH from 7.0 up to 7.51 leads to higher yield percent (91.3, 92.5 and 94.3%) for ethanol, kerosene and gas oil respectively, but greater than 7.51 the percent is decreasing.
- High concentration of both kerosene and gas oil results in higher yield percent of SCP up to a certain concentration of petroleum fraction, depending on nature of the crude oil, or amount of *n*-alkanes in gas oil or kerosene, where the increasing in *n*-alkanes leads to the biomass yield.
- High concentration of ethanol results decrease in the yield percent, because, the high concentration inhibits the speed reaction of biological growth.
- The optimum conditions of SCP production were 7.36 pH and 1.31% concentration for ethanol, and 7.42 pH and 4.0% concentration for kerosene and 7.51 pH and 4.0% for gas oil.
- It has been demonstrated that (*Bacillus subtilis*, and *Candida sp.*) can be used as a model organisms in SCP production, where considered nonpathogenic organisms.
- It be observed that use of *candida sp.* more economic than *bacillus subtilis*.
- The crude protein percent was complied to the general qualifications reported by previous researcher, where it was (61.25%).
- The obtaining (83.5%) of successful chicken embryos, allow to use the product (SCP) in animal feed as supplementary matter.

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**Table (1) Results of Bioassay of Chicken Eggs**

Dose No.	Gas oil Conc.(v/v)%	B	C	D	Absolute Killing %
1	0.5	2	1	10	11.1
2	1.0	1	3	10	28.6
3	2.0	1	1	10	0.0
4	3.0	2	3	10	14.3
5	4.0	1	3	10	28.6

B= number of killed embryos due to treatment, C= number of killed embryos without treating.

D= total number of eggs used in each experiment, Average killing %= 16.5%, Successful eggs %= 83.5

**Table (2)The Coded and Real Values of The Single Cell Protein Yield**

Using Central Composite Design Method

Exp.No	Coded Variable		Real Variable		Y <sub>1</sub> %	Y <sub>2</sub> %	Y <sub>3</sub> %
	X <sub>1</sub>	X <sub>2</sub>	X <sub>1</sub>	X <sub>2</sub>			
1	1	1	3.5	8.7	51.6	87.0	88.0
2	-1	1	1.01	8.7	88.7	56.5	67.2
3	-1	-1	1.01	7.3	93.5	79.6	69.4
4	1	-1	3.5	7.3	76.2	89.4	79.6
5	1.414	0	4.0	8.0	46.9	92.6	94.3
6	0	1.414	2.25	9.0	76.3	69.8	70.0
7	-1.414	0	0.5	8.0	90.5	56.2	58.1
8	0	-1.414	2.25	9.0	65.1	76.1	71.6
9	0	0	2.25	8.0	81.3	72.3	90.2
10	0	0	2.25	8.0	81.3	72.3	90.2
11	0	0	2.25	8.0	81.3	72.3	90.2
12	0	0	2.25	8.0	81.3	72.3	90.2

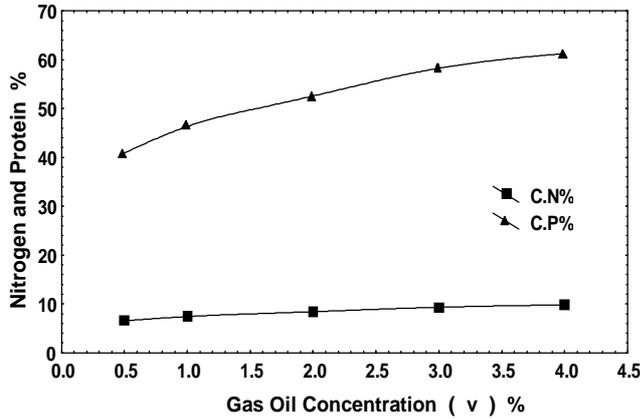


Figure (1) Effect of Gas Oil Concentration on Protein and Nitrogen Percent

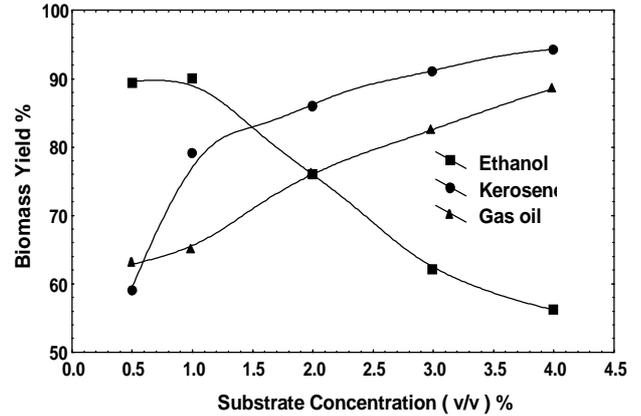


Figure (2) Effect of Substrate Concentration on Biomass Yield for *Bacillus Subtilis*

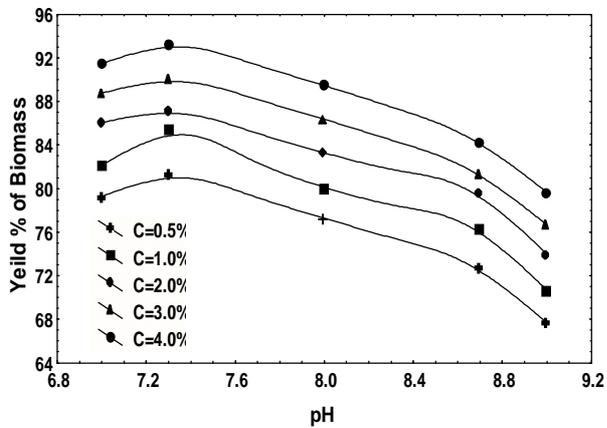


Figure (3) Effect of Substrate Concentration on Biomass Yield for *Candida sp*

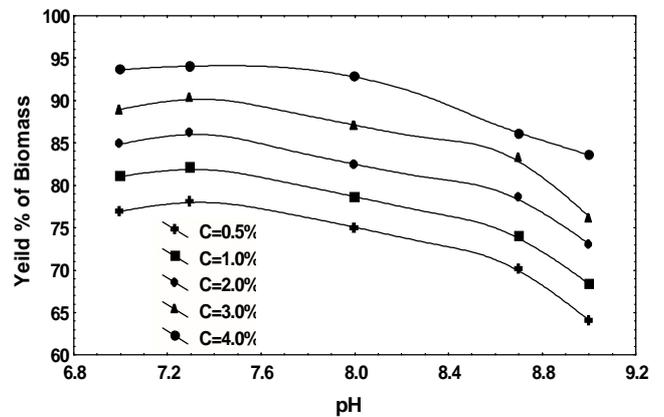


Figure (4) Effect of pH on Biomass Yield Using Gas Oil as Substrate

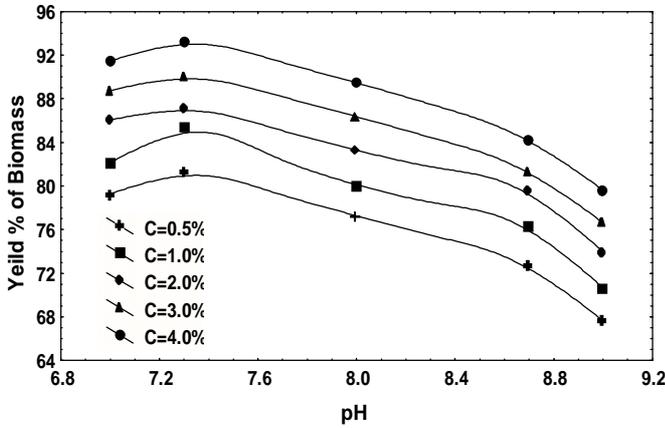


Figure (5) Effect of pH on Biomass Yield Using Kerosene as Substrate

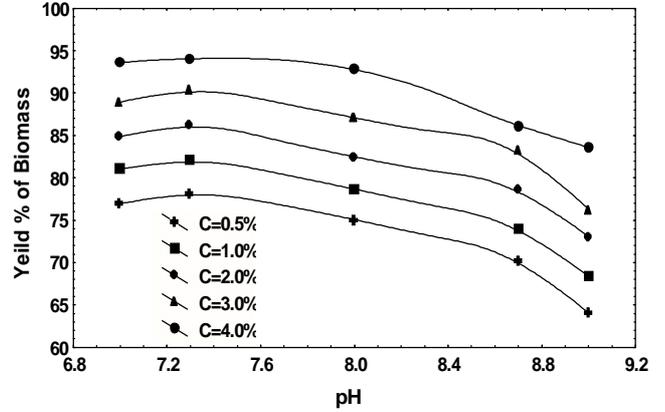


Figure (6) Effect of pH on Biomass Yield Using Gas Oil as Substrate

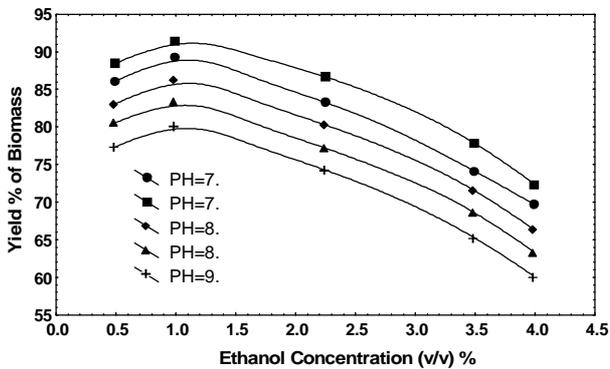


Figure (7) Effect of Ethanol on Biomass Yield at Different Values of pH

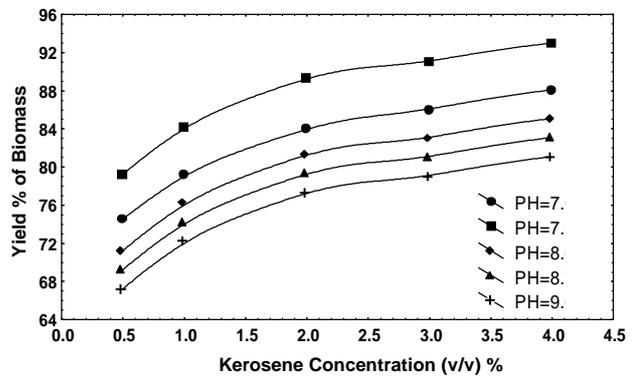


Figure (8) Effect of Kerosene on Biomass Yield at Different Values of pH

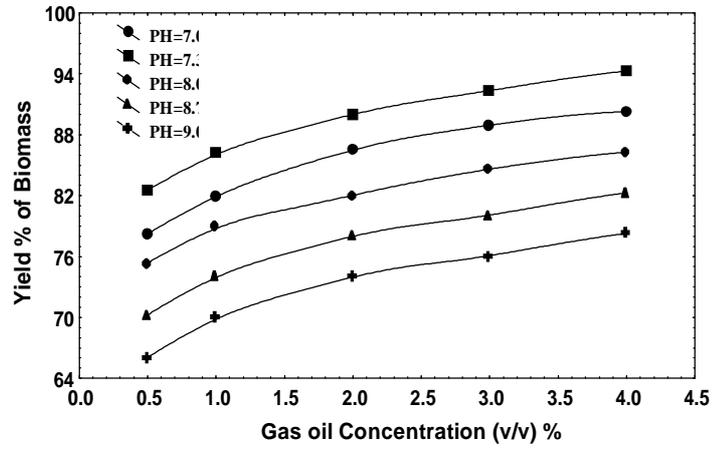


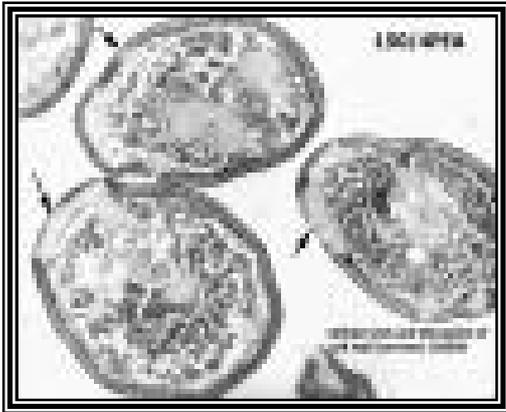
Figure (9) Effect of Gas Oil on Biomass Yield at Different Values of pH



Figure (10) *Bcillus Subtilis* Rods



Figure (11) Plat Agar of *Bcillus Subtilis*



**Figure (12) Candida sp. cells**



**Figure (13) Plat Agar of Candida sp.**