and high crude protein content at temperature 37°C, pH 6.8, mixing rate of 400 rpm and aeration rate of 1 v/v/m.

Bacterial culture give a maximum yield at dilution rate of 0.15 h -1. A similar observation has been reported for other methanol-utilizing bacteria [1].

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Disscussion

Protein content of isolated bacterial methylotrophs was measured. The GA16-1 and GA17 colonies were shown to be rich of protein. Biochemical tests revealed that the most of isolated bacteria were so-called faccultative methanol-utilizing bacteria assimilating methanol by the serin pathway [15]. The selection of a bacteria in second stage was based on growth rate on mineral salts medium with methanol as the only carbon and energy source and also protein content of biomass. The GA16-1 colony was the most suitable colony for the production of SCP on the basis of the growth rate, the cell yield and the protein content. The GA16-1 was found to be a gram negative bacillus.

Bacterial culture GA16-1 has relatively simple and predictable growth phases. The inoculated cells had a 2 h lag phase and it followed rapid growth at a uniform rate. The maximum growth rate was attained after 16 h when highest conversion of methanol to cell material was achieved.

It was found that the substrate concentration is an important factor affecting the growth of this bacteria [12]. The methanol concentrations within the range of 1-1.5% was optimum, The methanol concentration above 20 g/1 will inhibit the growth of these bacteria. These results agree with those of Abu-Ruwaida et al (1990).

Cultivation conditions such as: temperature, pH, dissolved oxygen and dilution rate were reported to affect the specific growth rate, biomass yield and accumulation of storage compounds [3, 8, 16].

The maximum yield of GA16-1 was at a dilution rate of 0.15 h^{-1}, beyond which the yield decreased. The crude protein content of cells was approximately same for various dilution rates tested.

Oxygen transfer and mass transfer of substrate to the cell are important factors for bacterial growth on either carbohydrates or hydrocarbons as carbon and energy sources [10, 12]. Agitation will greatly influence oxygen and mass transfer, therefore good agitation increases the growth of bacteria and cell yield. The rate of 400 rpm resulted the maximum cell yield. There is no increase in cell yield above and under 400 rpm. Under these conditions the crude protein of cells remained constant.

Aeration and oxygen transfer to the growing cell is also an important factor in bacterial SCP production under aerobic conditions, espesially from hydrocarbons, methanol and ethanol. The maximum cell yield and crude protein were obtained at aeration rate 1 v/v/m. With increasing aeration rate no more incrase in cell yield was observed.

Temperature tolerance is an important characteristic of bacterial strain for SCP production from hydrocarbons or alcohols. Cooling costs for bacterial SCP production can be significant unless high-temperature tolerant bacterium in the range of 35-45°C are utilized [5, 7]. The optimum temperature was about 37°C for the maximum cell yield and content of crude protein. The cell yield decreased with increases in the culture temperature.

The optimum pH for the growth of bacterial culture GA16-1 was 6.5-7.0. At this pH maximum cell yields and crude protein content of cells were maximum.

Conclusion

28 selected bacterial cultures were isolated from 17 sources by enrichment and plating cultures with methanol. The bacterial culture GA16-1 was selected to be superior in terms of the specific growth rate and cell yield. The results of batch culture revealed that a methanol concentration within the range 1.0-1.5% (w/v) produced highest growth, cell yield and high cellular protein content.

In chemostat optimization, cultivation conditions such as temperature, pH, oxygen, mixing rate and dilution rate have a profound influence on the growth rate, composition and the levels of bacterial intracellular metabolites, and will finally control the biomas yield coefficient. The two isolated bacterial cultures in our study had optimum growth, highest productivity ($Y_{x/s}$)

pH

The influence of pH of medium on the growth of bacterial culture GA16-1 was studied. The maximum cell yield and the content of crude protein at different pHs are shown in Figure (9).

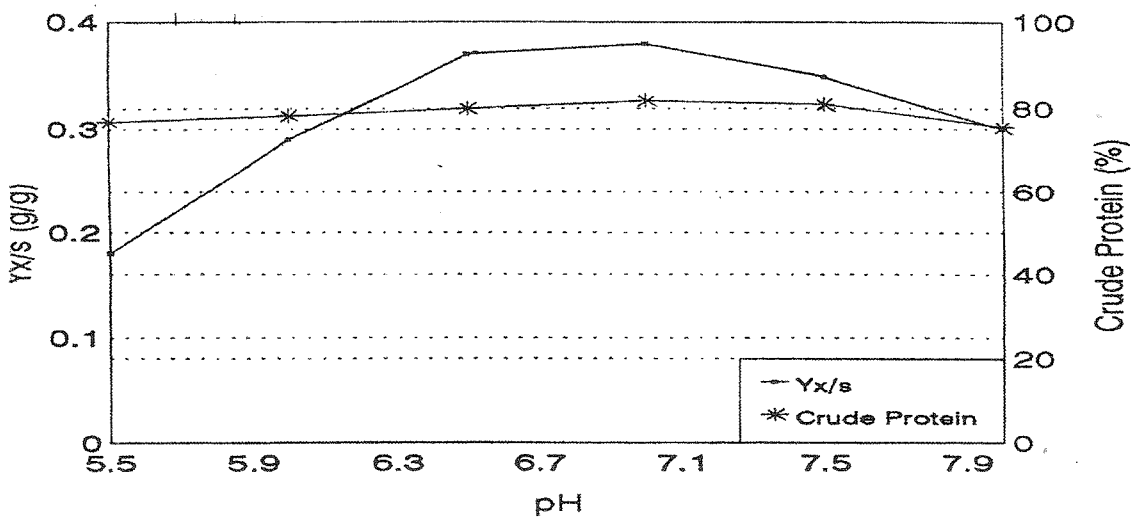


Figure (9) Effect of pH on the cell yield(■) and crude protein content of cells (*). Experimental conditions: Temp 37°C; impeller speed 400 rpm; D= 0.15 h^{-1}, pH: 7.0.

Aeration rate

The cell yield and crude protein content of bacterial culture GA16-1 at different aeration rates were studied and the results are shown in Figure (10).

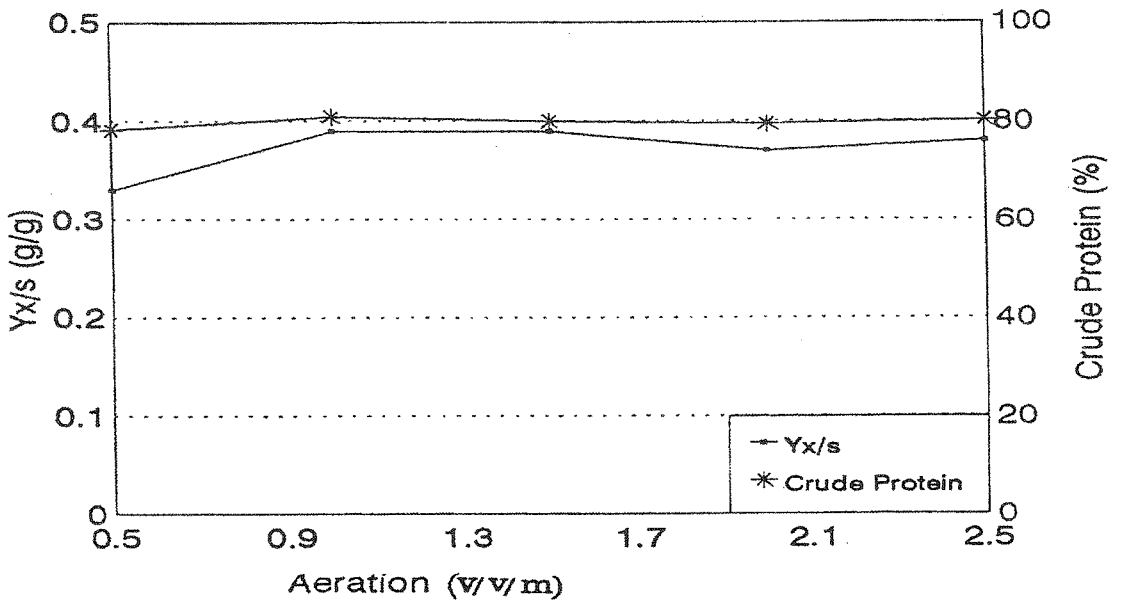


Figure (10) Effect of aeration rate on the cell yield (■)and crude protein content of cells(*). Experimental conditions: Temp: 37°C; pH 7.0; D= 0.15h^{-1}; impeller speed: 400 rpm

Impeller speed

An optimum yield of 0.33 gg^{-1} was obtained at impeller speed of 400 rpm.

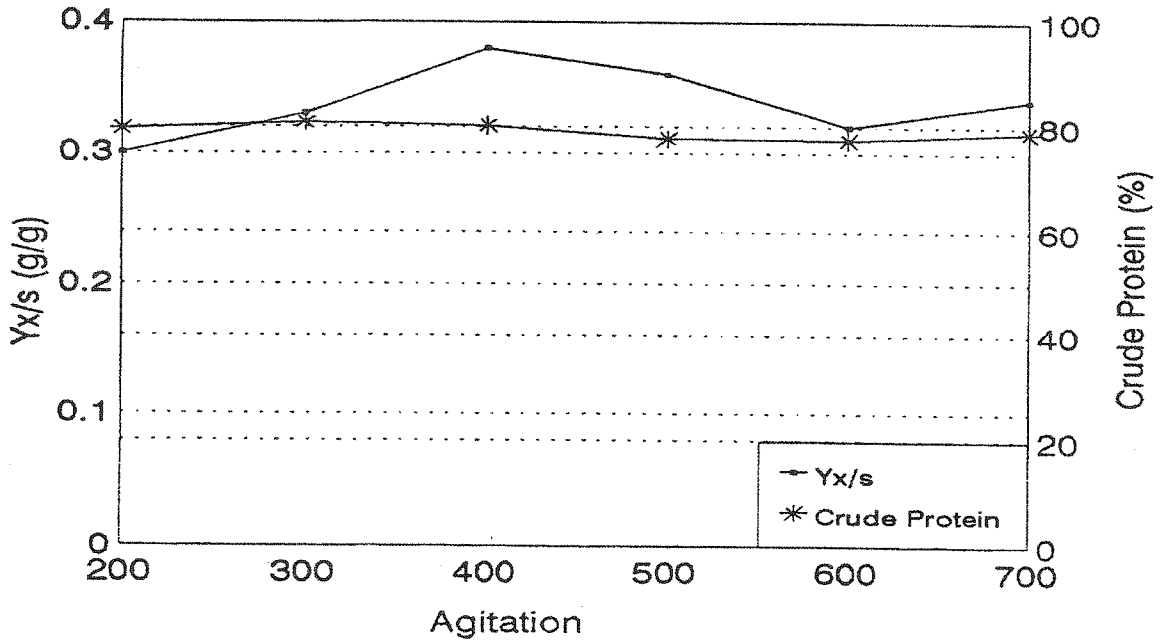
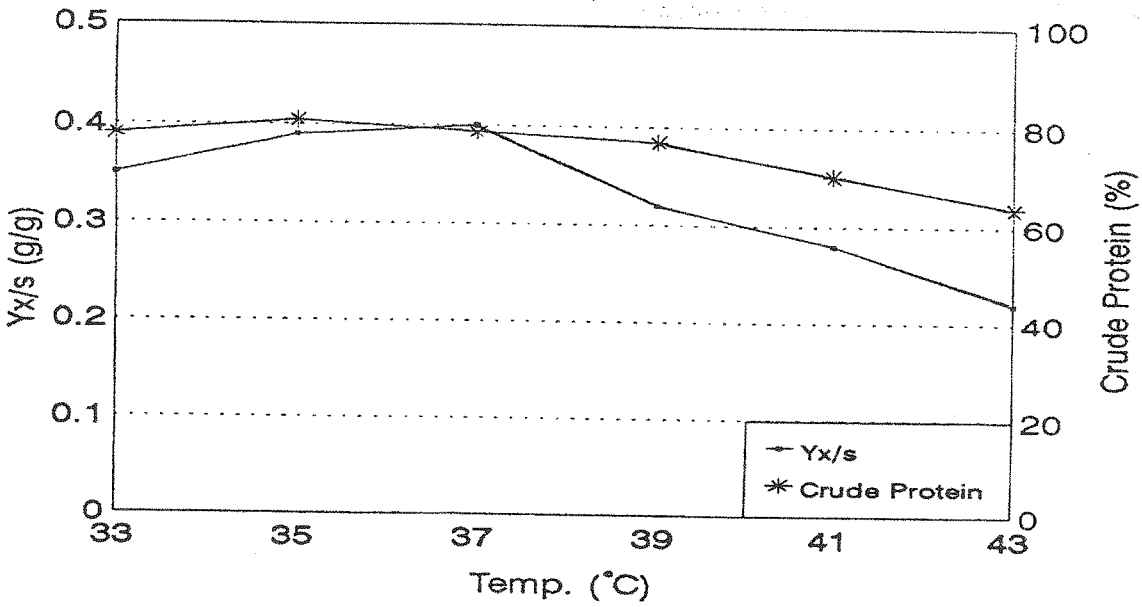


Figure (7) Effect of impeller speed on the cell yield (●) and crude protein content of cells (*). Experimental conditions: Temp: 35°C; D: 0.15 h^{-1}; pH: 6.8; aeration rate: 1 v/v/m.

Temperature

The maximum cell yield and contents of crude protein of bacterial culture GA16-1 was obtained with temperature is shown in Figure (8).



.Figure (8) Effect of temperature on the cell yield(●) and crude protein content of cells (*). Experimental conditions:Temp: 35°C; pH: 6.8; impeller speed 46 rpm; D = 0.15 h^{-1}; aeration rate 1 v/v/m

Methanol concentrations

The effect of various initial methanol concentrations on the ultimate optical densities was measured. The results are shown in Figure (4).

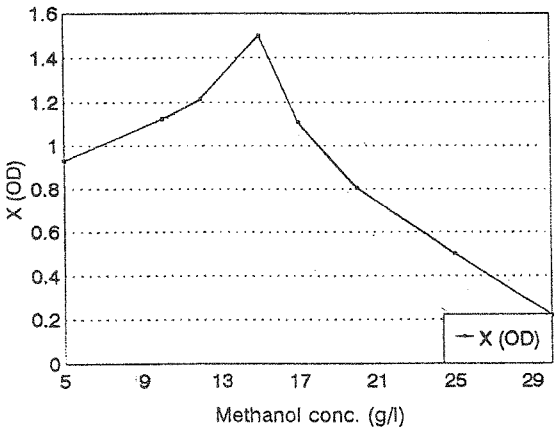


Figure (4) The effect of various initial methanol concentrations on the ultimate optical densities

Performance of bacterial culture under chemostat conditions

Bacterial culture GA16-1 was selected for investigations in chemostat conditions. The effects of dilution rate, agitation speed, temperature, aeration rate and pH were studied in chemostat.

Dilution rate (D,h^{-1})

Bacterial culture GA16-1 were cultivated in a chemostat under methanol-limiting conditions at different dilution rates. The cell yield and the content of crude protein at different dilution rates are shown in Figure (5) and (6).

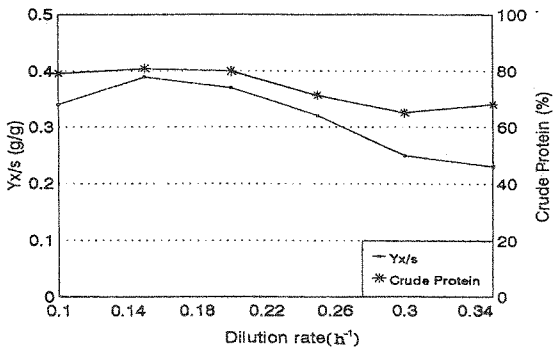


Figure (5) Effect of dilution rate on the cell yield and the protein content of cells (*). Experimental conditions: Temp.: 35°C; pH 6.8; impeller speed 300 rpm' and aeration rate 1 v/v/m

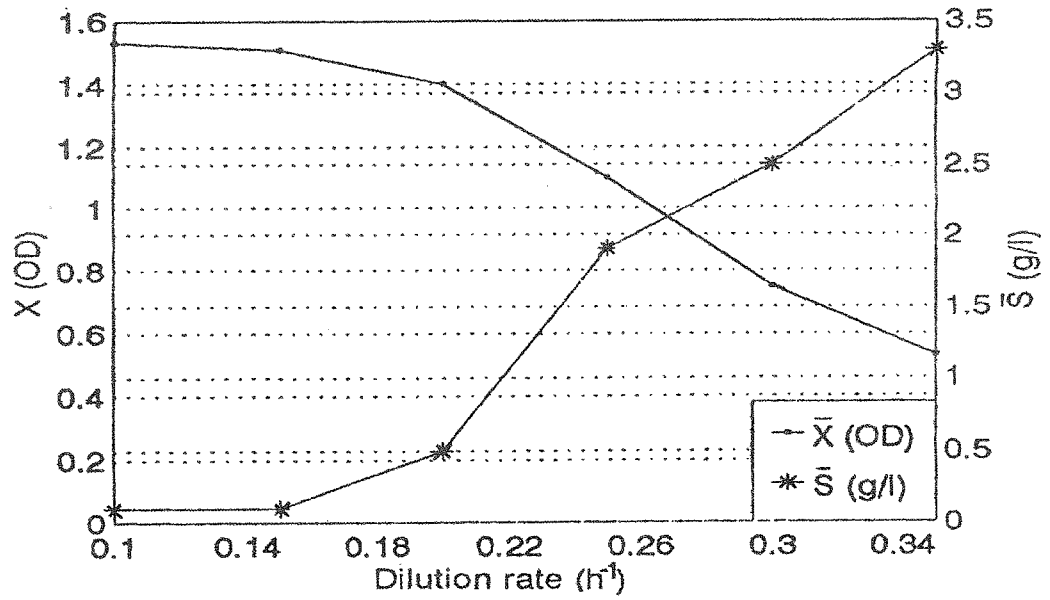


Figure (6) Effect of dilution rates on the cell density (OD) (●) and residual mehtanol concentrations (*). Experimental conditions: Temp.: 35°C; pH 6.8; impeller speed 300 rpm; aeration rate 1 v/v/m

Two methylotrophic bacterial cultures (GA16-1 and GA17) were selected for further experiments according to better growth and on the highest crude protein content.

Growth characteristics of two bacterial culture

Characteristics of two bacterial cultures were obtained in batch (flask) culture condition. The results of this experiment are shown in Figures (2) and (3).

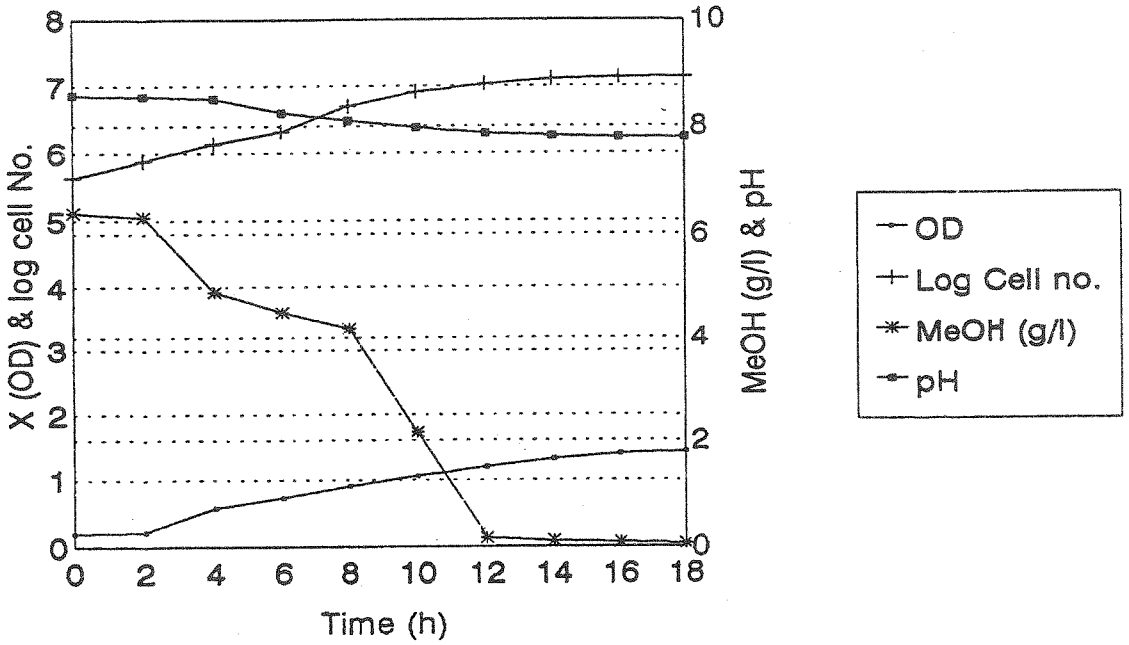


Figure (2) Growth characteristics of bacterial culture GA16-1 (m = 0.16h-1). Experimental conditions: Temp. : 35°C; pH 6.8; impeller speed 300 rpm; aeration rate 1 v/v/m

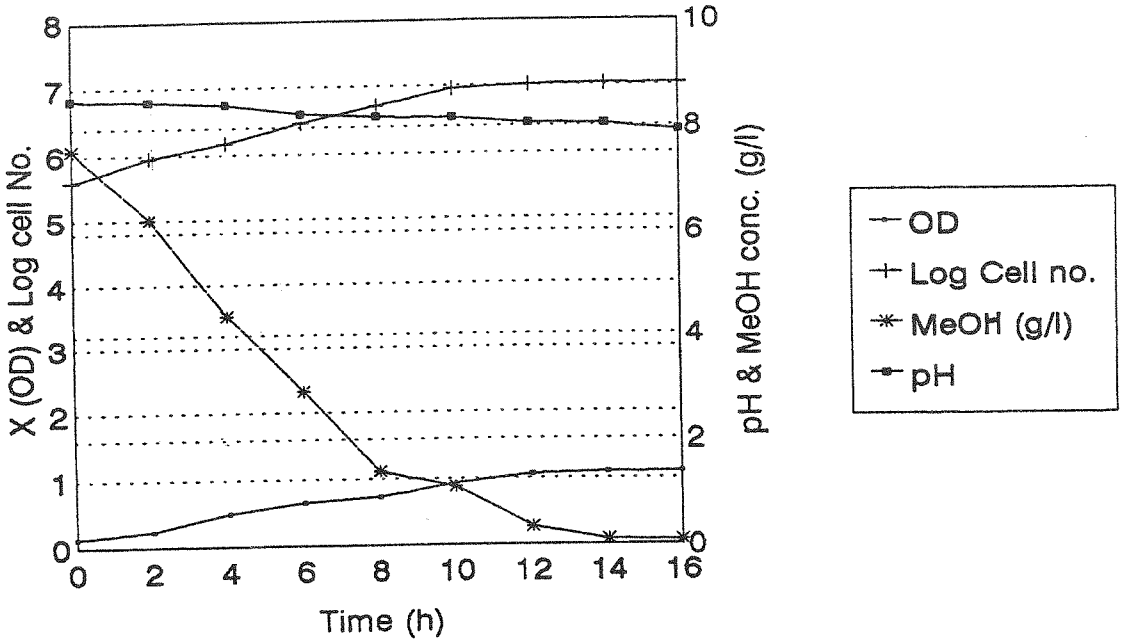


Figure (3) Growth characteristics of bacterial culture GA17 (m = 0.14h-1). Experimental conditions: Temp.: 35°C; pH 6.8; impeller speed 300 rpm; aera tion rate 1 v/v/m

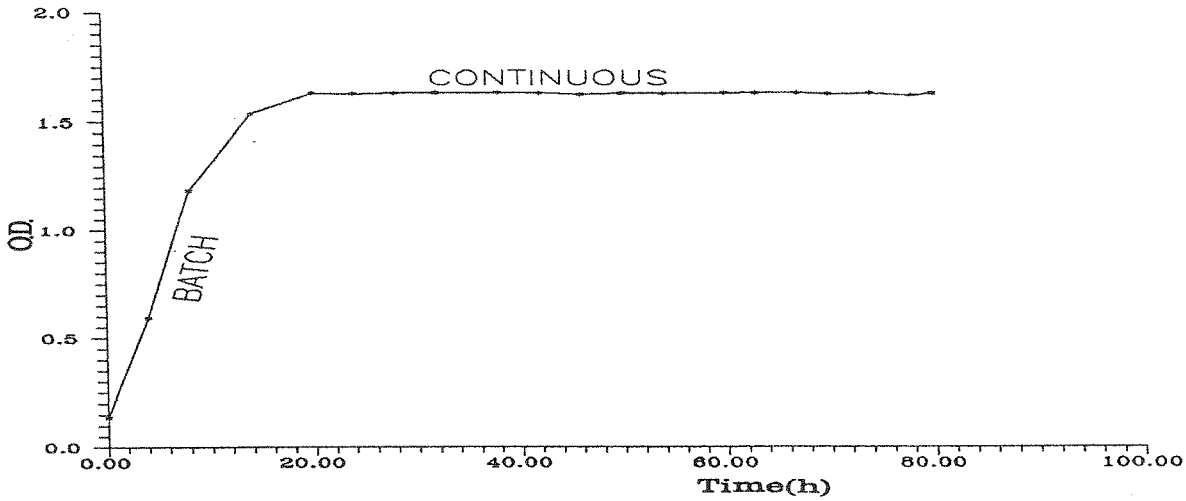


Figure (1) Steady - state in chemostat operation

Results

The selection of bacteria was based on good growth rate in mineral salts medium with methanol as the source of carbon and energy and bacterial protein content. The results of analyses of bacterial culture for percent of crude protein are shown in table (1) and (2).

Table (1) Analyses of isolated bacterial culture

Code of culture	Crude protein (%)	Code of culture	Crude protein (%)
GA17	80.8	GA13	50.2
GA16	46.8	GA503	64.3
GA501	78.6	GA4	44.2
GA502	51.0	GA82	48.4
GA15-1	47.0	GA14	52.9
GA2	48.2	GA162	80.0
GA16-1	75.5	GA10	49.7
GA15	58.0	GA171	83.2
GA173	73.3		

Table (2) Repeated analyses of selected isolated bacterial culture

Code of culture	Crude protein (%)	Code of culture	Crude protein (%)
GA17	79.5	GA173	66.6
GA16-1	78.9	GA162	77.5
GA502	55.4	GA501	73.3
GA171	72.5	GA503	48.4

phate, methanol concentrations and relative ease of achieving steady-state cultures in the continuous culture [8, 17].

Possible screening for methylotrophs may be done in locations of methane and methanol accumulation, i.e., marsh lands, oil drills, storage tanks, gas transport systems, animal manure deposits, municipal waste fills and natural ponds with boundary zones between aerobic and anaerobic conditions [8, 9, 17].

Materials and Methods

Microorganisms

Bacteria were isolated from 17 soil samples from oil-zones and distelleries waste water by the enrichment method with methanol.

Medium Preparation

The growth medium composition includes (in gl^{-1}): Na_2HPO_4 $12H_2O$: 5.51; KH_2PO_4: 1.40; $MgSO_4$. $7H_2O$: 0.60; $(NH_4)_2$ SO_4: 3.00; NaCl: 0.05; $CaCl_2$. $2H_2O$: 0.02; $FeSO_4$. $7H_2O$: 0.01; trace element solution: 0.5 mL. Trace element solution contained the following in gl^{-1}: $CoCl_2$. $6H_2O$: 4.00; $MnSO_4$. $4H_2O$: 12.10; $NaMoO_4$. $2H_2O$: 1.53; $ZnSO_4$. $7H_2O$: 3.20; H_3BO_3: 0.05. The pH of medium was adjusted to 6.8 ± 0.2 and 5g methanol was sterilized (0.2 mm filter) and added to the heat sterilized medium. Agar granule 1.2 - 1.5 (W/V) % was added to obtain solid medium for plate culture. This medium was used for the isolation, purification and maintenance of the methanol-utilizing bacteria.

Isolation and Purification

Isolation of methanol-utilizing bacteria were achieved by sample enrichment and then streak-plate method with gram staining technique. Isolation of these cultures resulted in 28 methylotrophic bacteria. Two isolates were selected for further investigation after batch culture tests (GA16-1 and GA17).

Preparation of Inoculum

Inoculum were prepared by transferring coloines of plate culture to 50 mL medium (MSB) in a 250 mL conical flask and then incubated on a shaker (200 r.p.m) for 20-22h at 37°C.

Batch Experiments

Conical flask (2 liter) containing 500 mL medium were inoculated with 50 mL inoculum and incubated under the above conditions. Samples were aseptically withdrawn at 2 h intervals to determine the optical density of the culture at 620 nm, cell count and methanol concentration. Since optical density is a direct measurment of the biomass concentration, the specific growth rate of the cultures was estimated from the logarithmic plots of optical density versus time during the exponential growth phase.

Chemostat Operation

Chemostat fermentation was carried out using 2-L fermentor. The fermentor were charged with the medium after sterilization and filter sterilized methanol (10 gl^{-1}). After achieving sufficient growth, continuous fermentation was started by feeding the nutrients at a dilution rate of 0.1 h^{-1} and substrate limitation of 10 gl^{-1} methanol. The temperature was maintained at 35°C and aeration rate at 1-1.5 v/v/m. The pH was maintained at 6.8± 0.2 by adding 1.0 N NaOH solution. Foaming was controlled by the addition of sterile silicone antifoam. When a steady state was achieved (Figure 1) samples of the fermentation broth were collected to determine optical density (OD), residual methanol, dry weight and cellular component of crude protein.

Bacterial cells were harvested by centrifugation at 12000 rpm (Beckman). Crude protein and total nitrogen was estimated by a CHN analyser (Heraeus, CHN-O-Rapid). Methanol concentration was determined by injection of 0.5 µl of sample to gas-liquid chromatography (Philips, PU4410) using a packed bed column (OV225).

Single-Cell Protein (SCP) Production from Methanol-Utilizing Bacteria in I.R. IRAN

S.A. Shojaosadati

Biotechnology Group, Chemical Engineering
Dept. Tarbiat Modarres University

M.N.Ghazali

Biotechnology Group, Chemical Engineering
Dept. Tarbiat Modarres University

Abstract

28 Colonies of methylotrophic bacteria were isolated from soil samples of oil-field area and waste waters of oil distilleries and selected for Single-Cell Protein production. Siginficant criteria for selection of bacteria were: substrate yield coefficient, specific growth rate and protein content. The continuous culture technique under chemostat was used to optimize the growth conditions of bacterial culture GA16-1. Optimal concentrations of methanol (10 g/l) as well as optimal growth parameters such as: pH (6.8), temperature (37° C), aeration rate (1 v/v/m), agitation speed (400 rpm) and dilution rate (0.15 h^{-1}) were maintained, the maximum biomass yield coefficient ($Y_{x/s}$) obtained under optimized conditions was 0.41 gg^{-1}.

Introduction

Single-Cell Protein production was proposed as a way to provide an alternative source of protein that could supplement the conventional sources of supply for the manufacture of compounded animal feeds [2, 6, 7, 8]. The incentive for SCP production is to be found particulary strong in Europe, Russia, Japan, Middle East and Africa; where indigenous agriculture is unable to provide the necessary protein-rich raw materials for incorporation into compounded animal feeds [4,13]. It is envisaged that SCP can provide a possible key for economic livestock production in some of these regions [2]. In the Middle East, specially, availablitiy of methane and methanol and their low price are exciter for SCP production from methanol.

Biosynthesis of protein from hydrocarbons and methanol by bacteria and yeast have been investigated. Methanol has many advantages compared with other, such as; it's solublity in water, free from polycyclic aromatic compounds, the explosion risk during production is minimal and it is partially oxidated and available in region. Handling of methanol is also easy and safe in transportation and during microbial biosysthsis [8, 11, 14, 18]. The methanol route for SCP production has already been developed in a number of establishments such as the ICI (U.K.) and Hoeschst/Uhde (Germany) [4, 7, 8].

In search for good methylotroph to be utilized for SCP production on a large-scale, one might search for a wide variety of features. These include a rapid growth rate, a high protein content (60-80% of dry matter), a good amino acid distribution, broad tolerance to pH, temperature, phos-