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Continuous Alcohol Production with Immobilized Growing Microbial Cells

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ABSTRACT. Growing cells of *Saccharomyces cerevisiae* immobilized in calcium alginate gel beads was employed in fluidized-bed reactors for continuous ethanol production from cane molasses and various sugar sources.

Some improvements were also made against microbial contamination and for the maintenance of yeast viability for a stable long run operation.

A pilot plant of 4 KL total column volume was constructed and it was affirmed that 8.5 v/v% alcohol was constantly produced from diluted cane molasses for over three months.

The productivity of this process was estimated at 0.6 KL ethanol/KL-fermentor, day with 95% of conversion yield against theoretical value.

The world's diminishing energy supply has created a growing anxiety about fuels, forcing industrial countries including Japan to strive for required quantities of energy, and in their all-out efforts for this purpose, to develop various kinds of alternative energy products to replace petroleum fuels.

Under these circumstances, Research Association for Petroleum Alternatives Development (RAPAD) in Japan was established in May 1980, getting support from the Ministry of International Trade and Industry (MITI). RAPAD is organized by 23 member companies from such industries as petroleum, fermentation, chemical and engineering. It inaugurated research activities in June 1980, taking charge of a national project under a 7-year program (1980-1986). The amount of investment required for the project is estimated at around 38.8 billion yen (US$ 150 million).

Table 1. Research activities of RAPAD.

1. Technology for manufacturing synfuels from syngas
2. Technology for upgrading tar sand bitumens and shale oils
3. Technology for biomass conversion and utilization

Research activities of RAPAD cover the following three fields as shown in Table 1. The subjects on biomass utilization are as follows.

(1) Technology developments utilizing cellulose for ethanol or butanol with physical or chemical pretreatment.

(2) Technology for continuous ethanol fermentation using immobilized yeast cells.

(3) Probing into other related items, recovering ethanol etc.

Studies also being advanced on problems which may arise when biomass alcohol is used in a mixture with gasoline as motorfuel.

'Continuous production of alcohol by immobilized yeast cells' is one of main themes of the biomass utilization project of the RAPAD. This work has been carried out by two groups in RAPAD since June of 1980 in a schedule to be completed in March, 1983. Two groups in the RAPAD have been engaged in the exploitation of this immobilized yeast process, the one is the use of specially designed artificial polymer (*i.e.* Photocrosslinkable polymer) and the other is the utilization of natural and/or synthetic materials as yeast carriers. The latter type of immobilization is the target of our group.

Several techniques have been proposed for continuous ethanol fermentation and some are applied to industrial production. A bioreactor composed of immobilized growing yeast represents a new trend in these techniques for its high productivity and for lowering the initial investment and operational cost of the alcohol fermentation process.

The approaches for the project were made as follows.

(1) Selection of carrier for immobilization of yeast cells and studies on the method of immobilization.

(2) Design of reactor and shapes of carrier.

(3) Selection of the most suitable strain for continuous ethanol production.

(4) Prevention against contamination.

(5) Studies on the maintenance of yeast viability.

(6) Scale-up to pilot plant.

Selection of Carriers

Living yeast cells were entrapped with various polymers (Kierstan and Bucke 1977 and White and Portno 1978). Various polymer beads containing living yeast cells were replaced separately in glass columns, and cane molasses solution was fed continuously in each column for 5 days.

Figure 1 shows daily changes of alcohol productivity of those columns. Carrier activities increased with yeast growth inside carriers. Among the polymers examined, Ca-alginate showed the best result. Also, Table 2 shows the results of

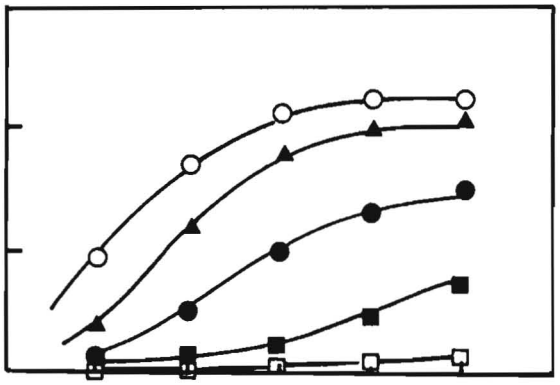


Fig. 1. Ethanol productivity of immobilized yeast entrapped in various polymers.
○ Ca-alginate gel; ▲ polyacrylamide gel; ● porous epoxy resin; ■ porous polystyrene; □ porous polyester microcapsule.

Table 2. Activity of various immobilized carriers.

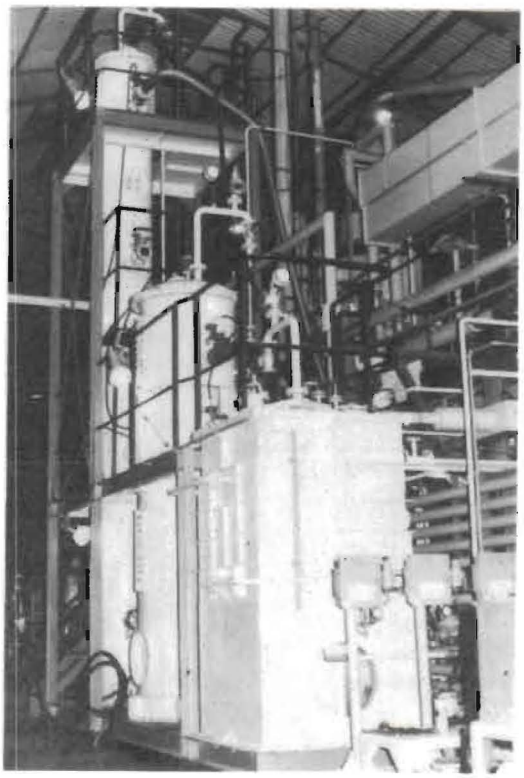
Immobilizing process	Alcohol producing activity	
	mg-alc/g-gel,hr	mg-alc/ml-gel,hr
Porous epoxy resin	26	15
Nylon microcapsule (mc)	2	1
Unsat. polyester mc	4	2
Acetyl butyl cellulose mc	14	8
Porous polystyrene mc	14	7
Polyacryl amide gel	36	20
Calcium alginate gel	40	22
Low methoxy pectin gel	35	19
Carrageenan gel	40	22
Agar gel	40	22
Silica sol	50	28

Table 3. Selection of supporting materials for microbial immobilization.

1. High carrier activity
2. Availability in quantity
3. Low cost of immobilization
4. Easiness of scale-up of operation
5. Mechanical strength for long life operation

various immobilization procedures. Entrapping methods with hydrogel possessed high activities. Carrageenan is comparable with calcium alginate, but it has undesirable characters during the large scale preparation and operation.

Calcium alginate was chosen as an entrapping material for its accessibility with these selection points as shown in Table 3.



Phot. 1. A picture of prototype reactors.

Design of Reactor

After bench scale studies, prototype reactors were constructed before pilot plant designation. The capacity of each reactor was one cubic meter. Photograph 1 shows these three reactors. One is the tall tower type reactor, L/D ratio can be adjusted from 7 to 10. The second is the short column type reactor, L/D ratio can be adjusted from 1.5 to 2. The last is the rectangular type reactor. The inside of this reactor was separated by many vertical plates and the reaction solution moves horizontally and snakewise from one end to the other.

According to the results of bench-scale experiments, a tentative process flow was decided as shown in Fig. 2. This process was designed to make the whole process operated aseptically. Photograph 2 shows the immobilized cell beads just after the preparation in a 1 KL-size reactor. Continuous preparation of immobilized cell beads with alginate was tried for the first time in a large scale, and conducted successfully without any trouble. Photograph 3 shows the immobilized cell beads during the pass of cane molasses solution. Good fluidization of beads was observed during fermentation.

Reaction characteristics were also studied for each column. The activities of immobilized yeast beads and productivity of each column was comparable, but it

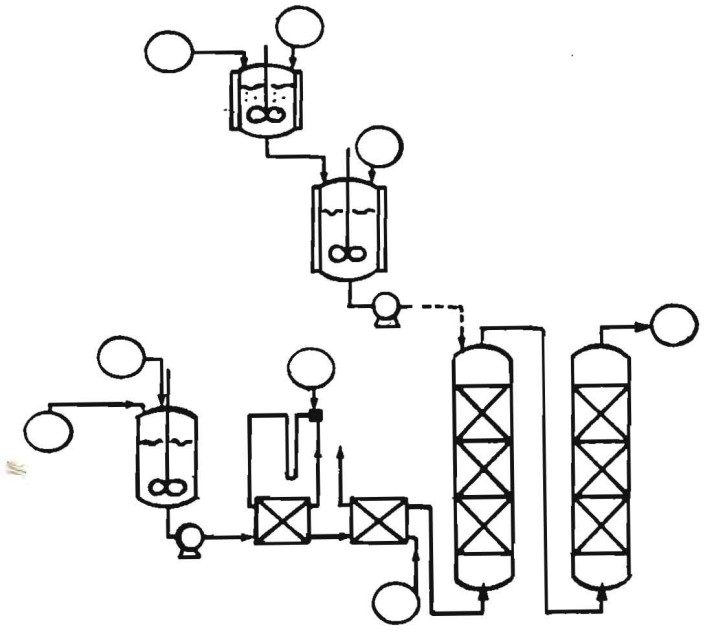
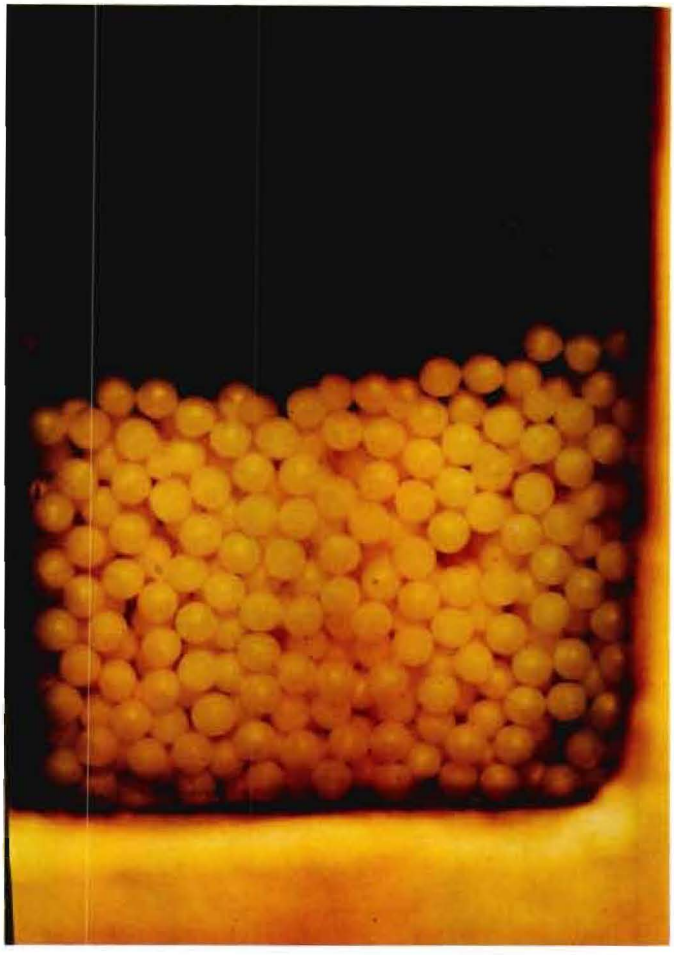


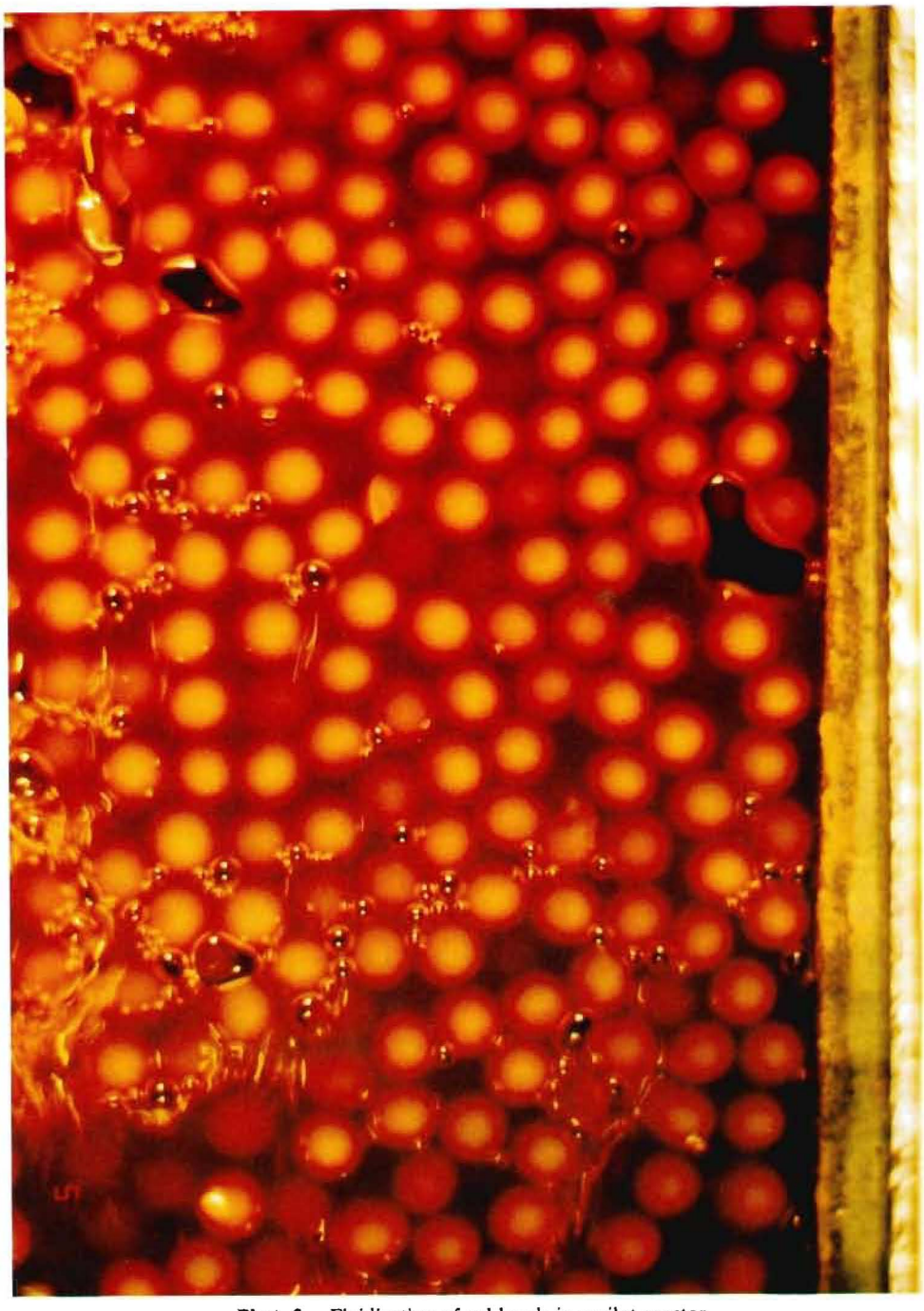
Fig. 2. A tentative process flow sheet (PROCESS-1).



Phot. 2. Immobilized cell beads just after the preparation in a 1 KL reactor.

was recognized that two columns at least must be connected in series to obtain higher conversion yield owing to the strong mixing effect of evolving CO_2 gas. Figure 3 shows the results of continuous operation of semi-pilot plant.

Sugar concentration of the inlet solution was about 15% and alcohol concentration of the outlet solution was about 8.5% (v/v). Conversion yield was more than 90%. And, productivity of alcohol was about 25g/L-gel, hr.



Phot. 3. Fluidization of gel beads in a pilot reactor.

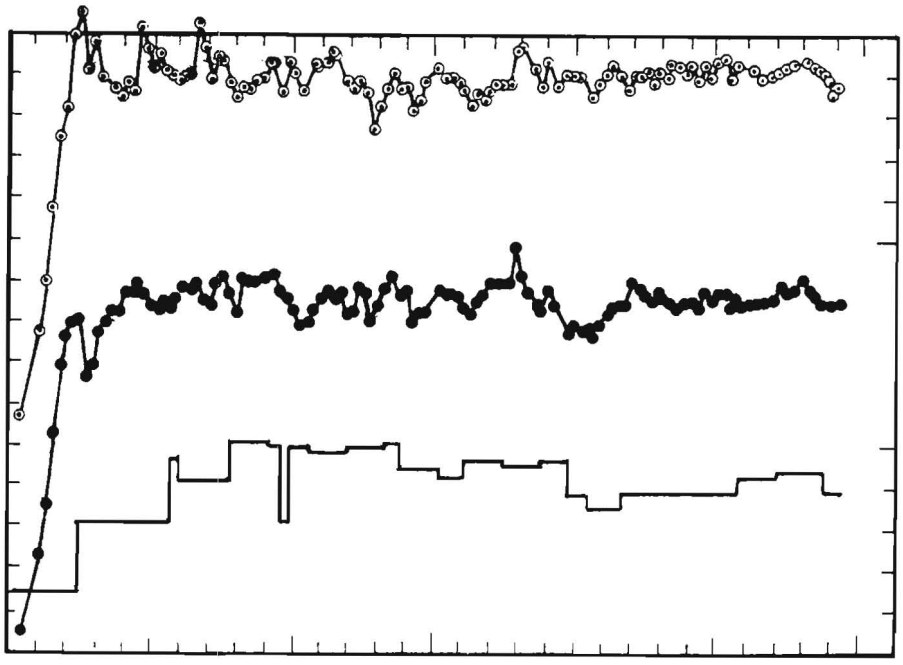


Fig. 3. Continuous operation in semi-pilot plant (1 KL columns in series, strain: T-29). Conversion yield ⊙, Alcohol conc. ●, Space velocity –.

Process Improvements

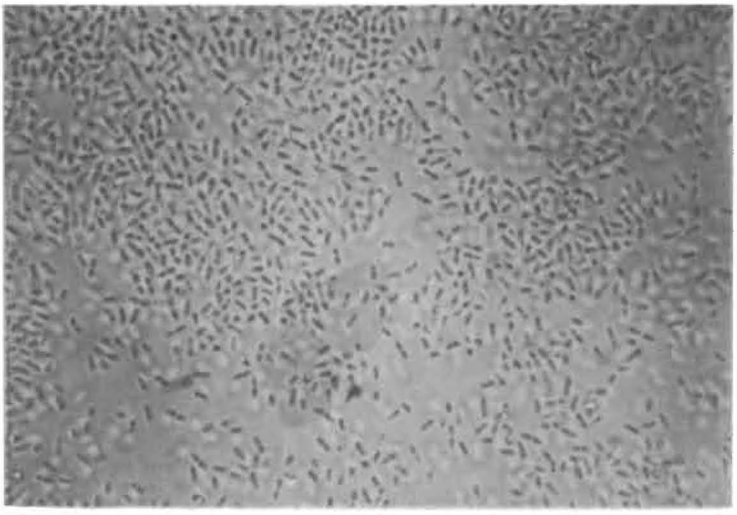
Prevention Against Contamination

In our experiments, sometimes operations were suffered by contamination by certain microbes. Photograph 4 shows one of the contaminant organisms classified as *Acetobacter*.

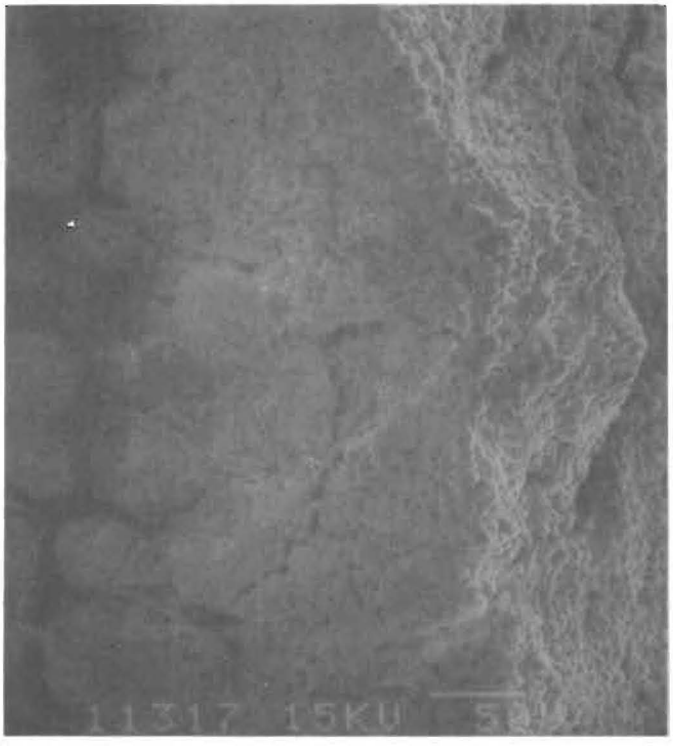
In order to obtain a good fermentation yield and make a continuous process stable for a long term, it is necessary to prevent a process from contamination. Studies on the isolated bacterial contaminants were made, and, as a result, operation at pH 4.0 with sulfuric acid or addition of some bactericidal substance to the inlet solution were also very effective. By employing such procedure, contamination problem was almost eliminated and now we can operate the process without pasteurization of the inlet medium.

Yeast Viability

It was recognized that the activity of immobilized yeast was gradually decreased during the bench-scale long-run column operations. For the stabilization



Phot. 4. A contaminant organism encoutered in this process.



Phot. 5. Scanning electron microscopic photograph of the tentative immobilized gel beads.

of long life operation, it should be noted in these approaches. Maintenance of yeast viability was also investigated.

Photograph 5 shows an electron microscopic picture of the cross section of an immobilized cell bead. Yeast growth was observed rich in outer layer than inside of the bead.

Yeast growth inside the carrier, which might be limited by substrate diffusion, was promoted by dissolved oxygen or certain sterols and unsaturated fatty acids which were required for yeast growth during fermentation (Andreasen and Stier 1954). Also, the entrapment of such supplements together with yeast into gel beads enhanced yeast growth and attained higher productivity (30 to 50g-ethanol/L-gel, hr). Moreover, aeration into reactor enhanced the operational stability of yeast growth near the surface of beads.

Photograph 6 shows a cross section of a gel beads prepared by new process. Yeast growth was observed abundantly around the gel.

Strain Improvement

An example of strain improvement is shown in Fig. 4. Moreover, 10% of alcohol production was attained continuously by the new strain. In this experiment,

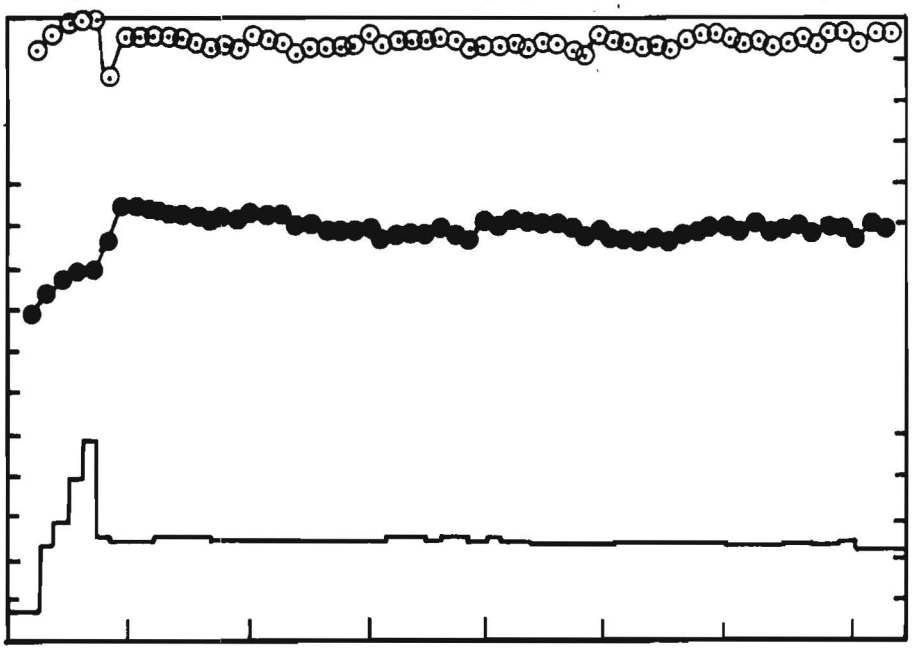
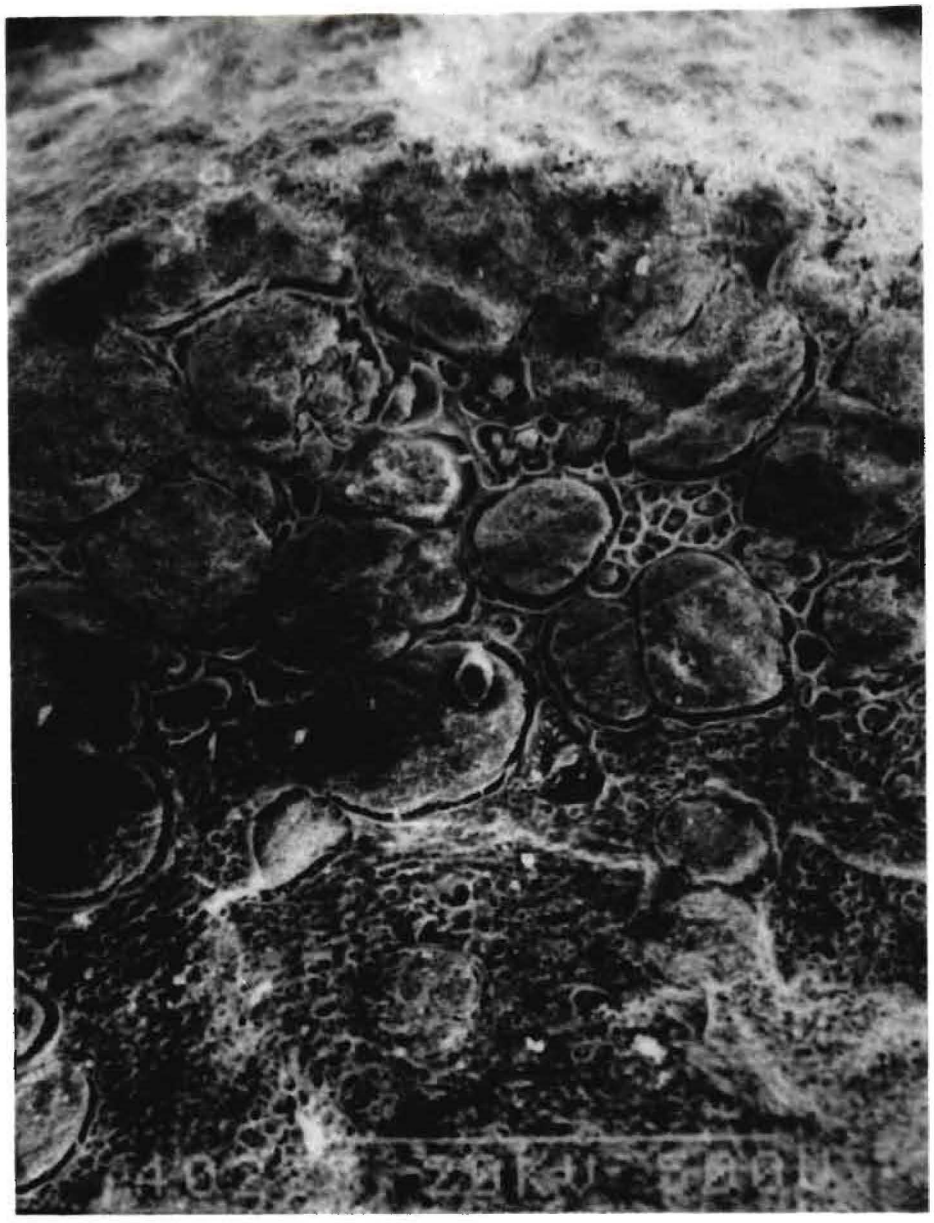


Fig. 4. Continuous operation in laboratory for higher concentration of ethanol (200 ml columns in series, strain: T-620).
Conversion yield ⊙, Alcohol conc. ●, Space velocity –.



Phot. 6. Scanning electron microscopic photograph of a gel beads prepared by new process.

all know-how hitherto obtained were applied and the productivity was increased to two-fold comparing with the tentative process. Also, cane juice or starch hydrolysate were good raw materials for higher concentration of alcohol fermentation.

Pilot Plant Operation

Figure 5 shows the new process. Now, we can operate without pasteurization of medium. And seed fermentor are not required for full scale operation of over 100 cubic meter reactor. Our principle in these developments is simplification of the total system. According to the results of semi-pilot plant operations, final pilot plant equipped with five column reactors was constructed in March, 1982. This pilot plant is composed of two channels of reactors, with one three columns and the other with two columns, both channels are connected in series. The reactor volume of each channel is 2 KL in full capacity, so the total column volume is 4 KL. Photograph 7 is a whole picture of our pilot plant. Pilot plant operation has been carried on since April 1982 to confirm the results hitherto obtained. Figure 6 shows the result of pilot operation during these three months. As shown in this figure, the following results have been obtained.

(1) 8.5 v/v% of ethanol was constantly produced from diluted cane molasses (sugar concentration 14 w/v%) at space velocity 0.4-0.5 for over three months at 30°C without any trouble.

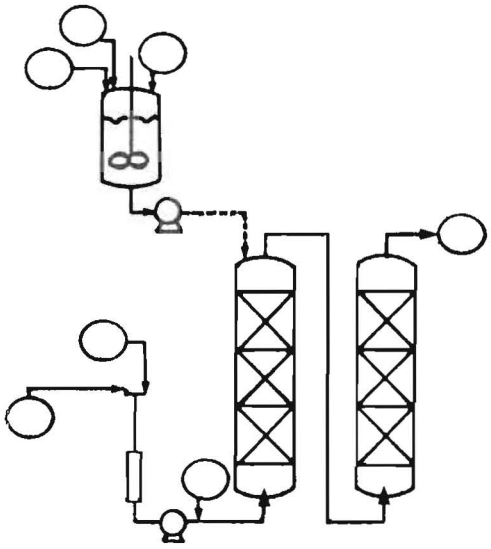


Fig. 5. Process flow diagram of the pilot operation.

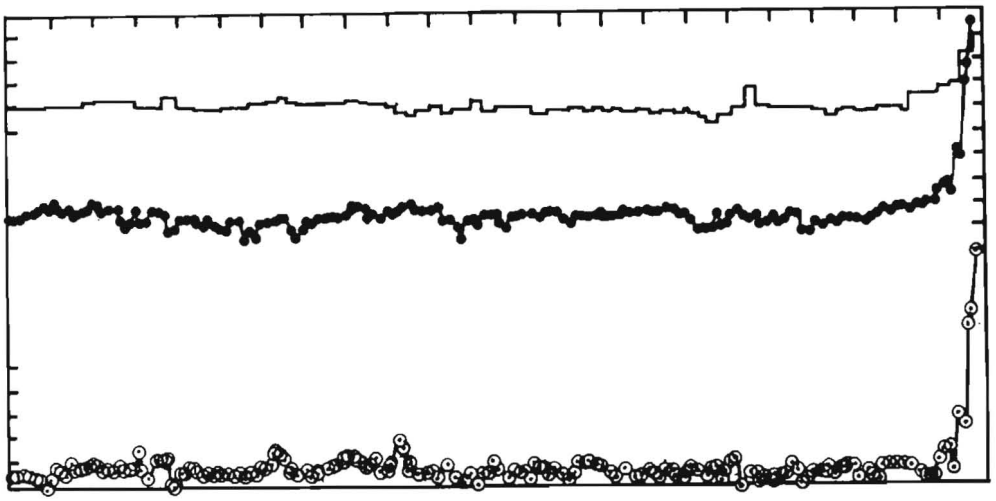
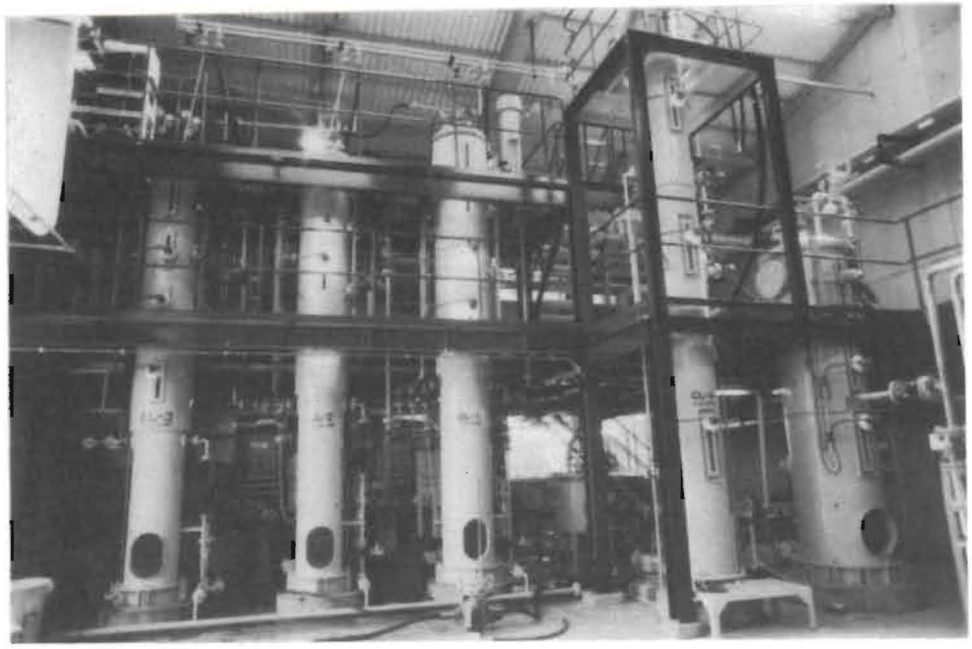


Fig. 6. Continuous ethanol production by immobilized growing yeast in the pilot plant. Conversion yield ⊙, Alcohol conc. ●, Space velocity –.



Phot. 7. Pilot plant of continuous alcohol fermentation by immobilized growing yeast cells (Hofu lab).

(2) The productivity of ethanol based on the total column volume (full capacity of reactor columns) was calculated as about 20 G/L/hr. This means that 600 liters per day of pure ethanol can be produced using 1 KL column reactor.

(3) Conversion ratio from sugar to ethanol was about 95% against theoretical yield.

This pilot plant is connected with computer system, and we are now aiming at a complete automatic operation of the plant. Photograph 8 shows computer display of the process. A minicomputer with software packages specifically developed for display and process control. Various confirmation tests of this process are now carrying on.

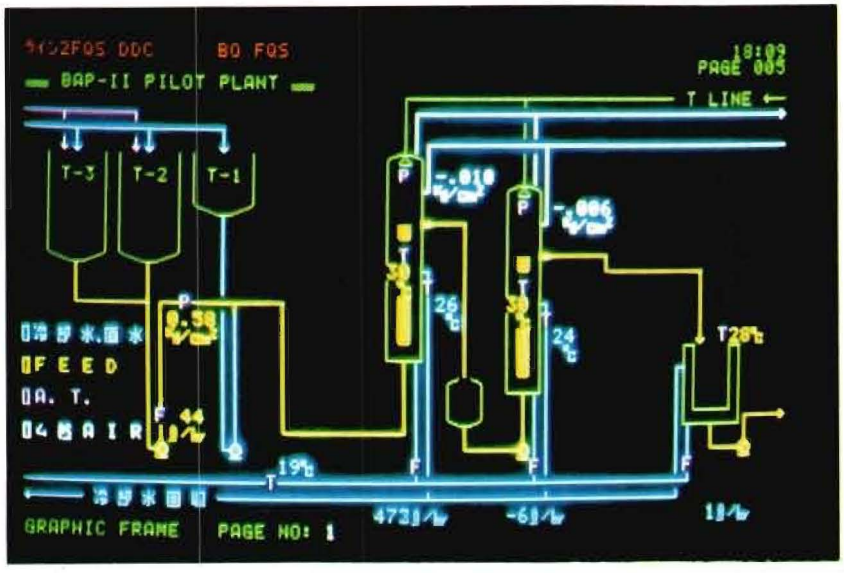
(1) Full automatic control, on line analysis of ethanol and sugar.

(2) Another process, easy handling with minimum control.

(3) Process available to various sugar sources.

Conclusion

Table 4 summarizes some advantages of our continuous fermentation process with immobilized growing yeast cells.



Phot. 8. Minicomputor display of the pilot plant.

Table 4. Some advantages of the immobilized growing yeast process.

1. Continuous fermentation with low operating and maintenance without skilful labor.
2. Low capital investment costs with higher productivity and higher yield.
3. Energy saving without sterile operation except gel preparation.
4. Applicable to a variety of liquid substrate.

This process is promising because of its high productivity, simple equipment and simple operation.

So this process may be industrialized on commercial scale in the near future.

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الإنتاج المستمر للكحول بوساطة الخلايا الميكروبية النامية المثبتة

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استخدمت خلايا خميرة *السكارروميسيس سيرفيسى* النامية والمثبتة على كريات جل ألجينات الكالسيوم فى مفاعلات خاصة لانتاج الكحول الإثيلى من دبس قصب السكر ومصادر سكرية أخرى.

وقد أدخلت بعض التحسينات لمقاومة التلوث الميكروبى وللابقاء على حيوية الخميرة فى عمليات التشغيل المستمر لفترات طويلة.

وقد أنشئت لهذا الغرض وحدة صناعية تجريبية ذات أعمدة سعة كل منها ٤ كيلولترات، أعطت كحولا بنسبة ٨,٥٪ حجم/حجم من دبس قصب السكر المخفف بشكل دائم لأكثر من ثلاثة أشهر.

وقد بلغت إنتاجية هذه العملية ٠,٦ كيلولتر من الكحول الاثيلى لكل كيلولتر من المخمّر يوميا، أى ٩٥٪ ناتج تحويل مقارنا بالقيمة النظرية.

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The Potentials of Yeast for the Production of Single-Cell Protein (SCP)

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ABSTRACT. Different local active yeasts were isolated from air, soils and muddy sludges. Potentialities and capabilities of 67 yeast isolates for formation of Single-Cell Protein (SCP) were evaluated following their cultivation on the fermentation medium containing diesel oil as the sole carbon source to select the most efficient oil-degrading yeast isolates. Identification of the most active organisms revealed that *Candida tropicalis* and *Yarrowia lipolytica* were the most efficient yeast strains having potentialities for SCP production. Biochemical changes which occurred during the fermentative production of SCP by *Candida tropicalis* revealed that during the fermentation process, a drop in pH towards the acid side was recorded, and this could result from the formation of certain organic acids during the initial stages of fermentation. Yield of yeast cell biomass increased with fermentation time, reaching an optimum at 168 hr, after which a decline in SCP was obtained. High diesel oil concentrations in the fermentation medium depressed yeast growth, and consequently a decrease in cell biomass was achieved, while low diesel oil concentrations, especially in the range of 40-60 ml/liter gave high titres of SCP. Addition of diesel oil at different intervals of the fermentation process promoted the yield of SCP.

The production of Single-Cell Protein (SCP) from various non-carbohydrate materials such as *n*-paraffins, ethanol and methanol has been reported as possible sources for food and feed material in the future. The capabilities of certain yeast to utilize petroleum products has attracted the attention of research workers to possibly synthesize proteins from petroleum sources. The commercial production of yeast by fermentation on certain hydrocarbons is now an acomplished fact (Tawfik *et al.* 1981).

Nishimura and Ueda (1973) reported that the genera *Trichospora* and *Torulopsis* propagated on media containing at least one of C_{5-9}polyhydric alcohols at the major carbon source. Chepigo *et al.* (1973) stated that the treatment of petro-

leum distillate containing 98% *n*-alkanes (C_{11}-C_{18}) with certain nutrients followed by yeast cultivation resulted in production of high protein yield. Tsao (1976) used hydrocarbons as carbon source in the fermentation medium for biosynthesis of SCP. Kaemmer (1974) reported that alkane yeasts grown on gas oil and *n*-paraffins respectively contained 69.0 and 65.5% crude proteins with 1.09 and 1.17% L-methionine, and these levels were higher than those of brewer's and feed yeast. Hong (1976) reported that more than 580 methanol utilizing yeasts were isolated from samples collected throughout South Korea. Two strains showed good biomass yield and were tentatively identified as *Candida melinii* and *Rhodotorula glutinis*. Suzuki (1977) published that the production of yeast and bacteria on hydrocarbon substrates were acceptable terms of nutritional value and toxicity. Suzuki *et al.* (1977) stated that *Candida paraffinica* was cultured on straight-chain hydrocarbons and produced high protein containing cells. Litchfield (1977) gave a report on the production of SCP from gas oil and *n*-paraffins. Miura and Yo (1974) reported the uptake mechanism of liquid hydrocarbons of low solubility in water using microorganisms with different affinities for liquid hydrocarbons. Micro-organisms which could utilize hydrocarbons were much more adherent to hydrocarbons than those which could not. The adhesive force between *Candida intermedia* IFO 9761 and hydrocarbon was higher than that of *Candida tropicalis* ATCC 2036, although both could utilize hydrocarbons. The total hydrocarbon uptake from both the drop and accommodation forms of hydrocarbons was much higher than that from dissolved hydrocarbons. Kasymova *et al.* (1978) stated that when *Candida lipolytica* and *Candida curvata* were cultivated on a mineral medium containing various hydrocarbon fractions of gas condensate (dearomatized fraction, purified *n*-alkanes, *n*-paraffins, and *n*-paraffin-naphthene fractions, etc.), maximum growth and cell biomass yield was observed with the *n*-paraffin fraction.

The objective of the present study was to isolate local yeast strains from different environmental sources, and to study their potentialities for the utilization of local hydrocarbons for the production of SCP.

Experimental

The isolation of organisms was restricted to yeasts which utilize petroleum products as carbon sources for production of Single-Cell Protein.

Isolation Medium

To minimize nonhydrocarbon-utilizing yeast isolates, the isolation medium was prepared without carbon sources except petroleum products. The medium used in isolation of hydrocarbon-utilizing yeast strains contained the following ingredients (g/*l*): NH_4Cl, 2.0; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4 . 7H_2O$, 0.5; $MnSO_4 . 4H_2O$, 0.05; $FeSO_4 . 7H_2O$, 0.005, and the carbon source was in the form of diesel oil, 100 ml. The ingredients were mixed thoroughly in distilled water and made up to

1000 ml. The pH value of the isolation medium was adjusted to 5.5-6.0. The isolation medium was sterilized at 121°C for 15 min. After sterilization, the isolation medium was ready to be utilized for the isolation of yeast isolates which have potentialities for biological degradation of diesel oil. Diesel oil is a local hydrocarbon obtained from Jeddah Oil Refinery Company, Manufacturing Department (Jeddah-Saudi Arabia).

Sources of Yeast Isolates

The yeast isolates were obtained from different sources. The sources were air, soils and sludge. The sludge was obtained from Jeddah Oil Refinery Company, Manufacturing Department. Samples were obtained from gas stations, especially soils saturated with petroleum products near fuel pumps. These soil samples were collected in sterile Petri dishes, and used for the isolation of yeasts capable of utilizing petroleum products. The technique of isolation of yeasts was dependant on the origins or sources from which yeasts were isolated.

Maintenance Medium

The medium used to maintain the yeast strains contained the following ingredients (g/*l*): glucose, 10.0; peptone, 5.0; yeast extract, 5.0; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4 . 7H_2O$, 0.5; $MnSO_4 . 4H_2O$, 0.05; $FeSO_4 . 7H_2O$, 0.005; agar, 30.0 in 1000 ml distilled water. The ingredients were mixed thoroughly and the initial pH value was adjusted to 5.5-6.0 followed by digestion in a boiling water bath for 10 min. The medium was poured into test tubes (25 × 150 mm), containing 10 ml of medium. The tubes were plugged with cotton and sterilized at 121°C for 10 min. The tubes containing the sterile medium were used as slants for propagating the yeast isolates. The slants were inoculated with the yeast isolates under aseptic conditions, and incubated at 30°C for 72 hr to obtain luxuriant growth. The inoculated slants were kept as stocks in a refrigerator at 5°C.

Fermentation Studies

The fermentation process investigations were carried out to investigate capabilities and potentialities of the yeast isolation in the biodegradation and utilization of diesel oil for the biosynthesis of Single-Cell Protein.

Vegetative Mediumn

The vegetative medium used for growing the yeast isolates contained the following ingredients (g/*l*); glucose, 10.0; peptone, 5.0; yeast extract, 5.0; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4 . 7H_2O$, 0.5; $MnSO_4 . 4H_2O$, 0.05; $FeSO_4 . 7H_2O$, 0.005 in 1000 ml distilled water. The ingredients of vegetative medium were mixed thoroughly, and the initial pH value of the medium was adjusted to 5.5-6.0. The medium was portioned into Erlenmeyer flasks (250 ml capacity), each containing

50 ml of the medium. The flasks were plugged with cotton and sterilized at 121°C for 15 min. When the flasks attained ambient temperature, they were inoculated under aseptic conditions with a standard inoculum of the previous yeast strains. The inoculated flasks were incubated on a rotary shaker (200 rpm) at 30°C for 72 hr in order to obtain luxuriant growth. At the completion of the fermentation process, the vegetative medium was ready to be used as inoculum for the fermentation production of Single-Cell Protein.

Fermentation Medium

The fermentation medium used for the production of Single-Cell Protein contained the following ingredients (g/*l*): diesel oil (C_{12}-C_{20}), 40 ml; NH_4Cl, 6.0; KH_2PO_4, 2.0; KCl, 0.5; $MgSO_4$. $7H_2O$, 0.5; $MnSO_4$. $4H_2O$, 0.05; $FeSO_4$. $7H_2O$, 0.005 in 1000 ml distilled water. The ingredients were mixed thoroughly and the pH value of fermentation medium was adjusted to 5.5-6.0. The fermentation medium was portioned into Erlenmeyer flasks (250 ml capacity), each containing 50 ml of the medium. The flasks were plugged with cotton and sterilized at 121°C for 15 min. When the flasks attained room temperature they were inoculated under aseptic conditions with standard inoculum of the yeast strain. The percentage of inoculum was 1.0%. The inoculated flasks were incubated on a rotary shaker operating at 200 rpm at 30°C for 7 days. At the end of the fermentation process, the fermented media flasks were harvested and the final pH value of the fermented medium was determined. Dry weight of growing yeast cells, and total protein percentage present in dry cell biomasses and fermentation broths were determined.

Biochemical Changes During the Production of Single-Cell Protein

The fermentation medium containing the previously mentioned ingredients was prepared and the pH was adjusted to 5.5-6.0. It was portioned into Erlenmeyer flasks (250 ml capacity), each containing 50 ml of the medium. The flasks were plugged with cotton and sterilized at 121°C for 15 min. When the flasks attained room temperature, one ml of sterile diesel oil was added under aseptic conditions into Erlenmeyer flasks. The flasks were inoculated with a standard inoculum of active strain of yeast isolate. The percentage of inoculum was 1.0. The inoculated flasks were incubated on a rotary shaker operating at 200 rpm at 30°C for different time periods, and every 24 hr, five flasks were harvested and the final pH value of the fermented medium, dry cellular biomasses, total cellular and culture filtrate proteins were determined.

Effect of Diesel Oil Concentration on Formation of Single-Cell Protein

The fermentation medium previously described was prepared having the same composition so described. The pH value of fermentation medium was adjusted to 5.5-6.0. The medium was dispensed into Erlenmeyer flasks (250 ml capacity), each

containing 50 ml of medium. The flasks were plugged with cotton and sterilized at 121°C for 15 min. After cooling, flasks were supplemented with different concentrations of diesel oil under aseptic conditions. The experimental concentrations of diesel oil (ml/litre fermentation medium) were: 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, and 100 ml. The flasks were incubated on a rotary shaker operating at 200 rpm for 168 hr. At the end of the fermentation process, the flasks were harvested and analysed.

Addition of Diesel Oil at Differnt Periods of the Fermentation Process

Flasks containing sterile fermentation medium were supplemented under aseptic conditions with 0.5 ml sterile diesel oil (0.5 ml diesel/flask), and inoculated with a standard inoculum of an active strain of yeast isolate. The inoculated flasks were incubated on a rotary shaker at 30°C. Every 24 hr of the incubation period, 0.5 ml sterile diesel oil was added aseptically into the fermentation medium for five days. Every 24 hr until the end of the fermentation process, five flasks were harvested and analysed.

Determination of Final pH Value

The final pH values of fermented media were determined using Corning Scientific Instruments, Model 12, Research pH-meter.

Determination of Biomass

Dry cell weight of the yeast isolates grown in fermentation medium containing diesels oil as carbon sources for production of Single-Cell Proteins was used to ascertain biomass. The fermented media were centrifuged to separate cell mass, and dried in an oven at 100-150°C until a constant dry weight was obtained. The filtrates were also used to determine total extracellular protein.

Determination of Total Proteins

The total protein present in dry cell biomasses and culture filtrates were determined by the reaction with Folin reagent (Lowry *et al.* 1951).

Results and Discussion

Yeast Isolates and Their Potential for the Production of SCP

A total of 67 yeast isolates was obtained in pure culture from air, soil and sludge sources. These microorganisms were related mainly to the generic nomenclature: *Candida, Torula*, and *Rhodotorula*. Yeast isolates were considered active if they grew on the medium containing diesel oil as sole carbon source for biosynthesis of proteins. When yeast isolates were cultivated in the fermentation medium

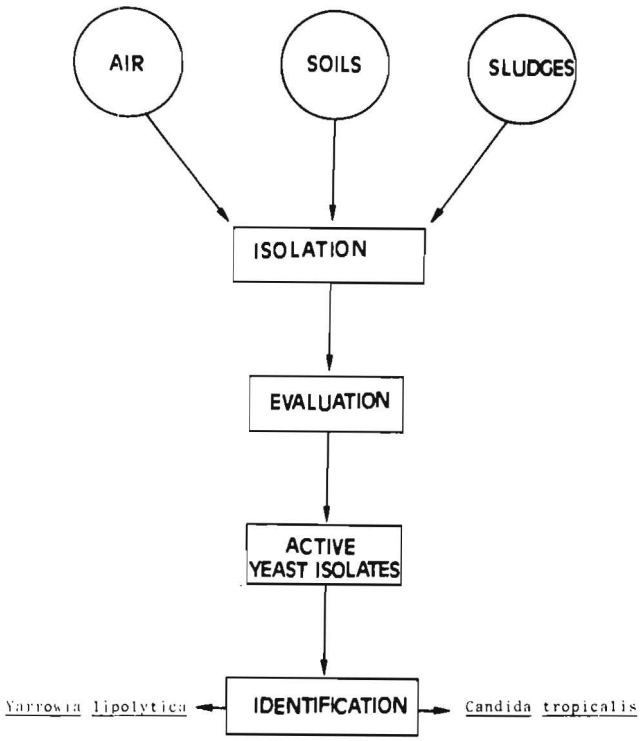

Fig. 1. Yeast sources, their isolation, evaluation and identification.

they exhibited a varying potential for the production of Single-Cell Protein. Their potential to degrade diesel oil were also different, and consequently the end-products represented as cell biomass were also variable. The most active yeasts isolated were selected for taxonomic identification (Fig. 1).

Identification of Active Yeast Isolates

The active yeast isolates were identified in Centraalbureau voor Schimmelcultures, yeast Division, The Netherlands. According to their identification, the most active yeast isolates were *Candida tropicalis* and *Yarrowia lipolytica. Candida tropicalis* was selected as the best SCP producer and was used in the further experiments.

Biochemical Changes Which Occurred During the Fermentative Production of Single-Cell Proteins

Candida tropicalis was grown in the fermentation medium containing diesel oil as sole carbon source for formation of Single-Cell Protein. The results obtained

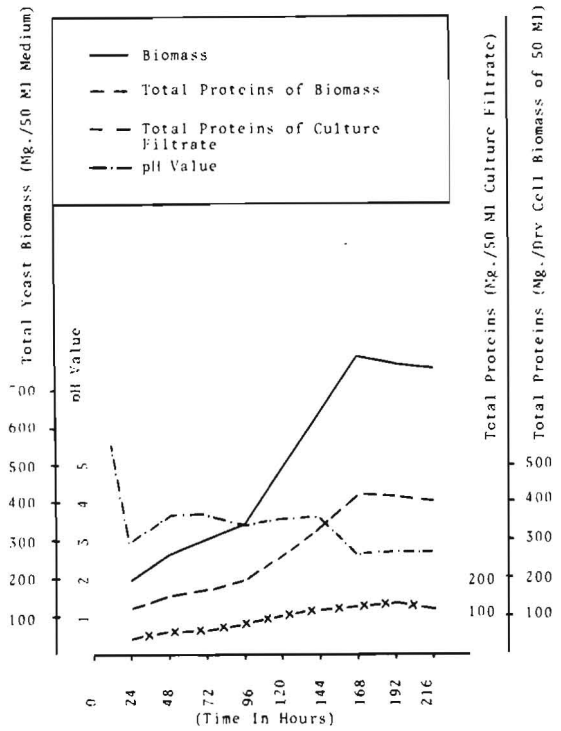

Fig. 2. Biochemical changes which occurred in the fermentative production of SCP by *Candida tropicalis*.

(Fig. 2) show that the initial pH of the fermentation medium was shifted towards acid side. The increase in acidity increased with the increase of incubation period of the fermentation process. The shift in pH value was arbitrary due to formation of organic acids and related metabolites resulting from yeast growth. Dry cell mass also increased with time reaching a maximum at 168 hr, above which a decline in cell growth was obtained. Total cellular protein was increased with time reaching its maximum at 168 hr, above which a decrease in total protein was obtained. The increase in yeast cell mass was associated with increase in total proteins during the different periods of the fermentation process. Total extracellular protein present in culture filtrates of the fermentation medium were small compared to cellular protein.

Role of Diesel Oil Concentration

Diesel oil was used in the fermentation medium as sole carbon source in formation of Single-Cell Protein. The results obtained (Fig. 3) show that diesel oil concentration affected SCP production. Yeast cell mass increased with the increasing

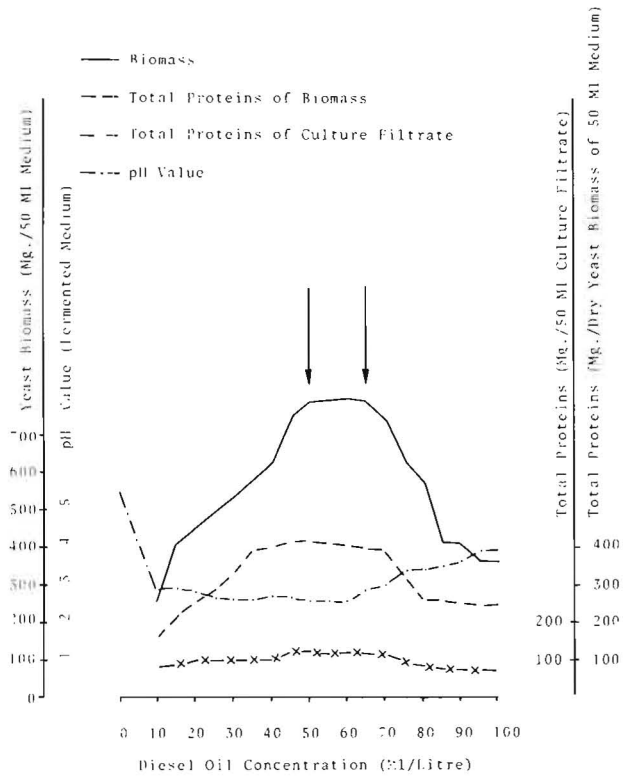

Fig. 3. Role of diesel oil concentration on formation of SCP by *Candida tropicalis*.

diesel oil concentration reaching its maximum at 45-50 ml/litre fermentation medium, above which a decline in yield of cell biomasses was recorded. The final pH of fermented medium shifted depending upon diesel oil concentration. Increase of yeast cell biomasses was associated with increase in total protein. High concentrations of diesel oil were harmful both to yeast cell growth and total protein formation. Optimal diesel oil concentrations were in the range of 40 to 65 ml per litre of fermentation medium.

Addition of Diesel Oil at Different Periods of the Fermentation Process

Addition of diesel oil at different incubation periods to the fermentation medium affected the fermentative production of SCP. The results obtained (Fig. 4) show that *Candida tropicalis* gave high titres of SCP when it was added into the medium at different periods of the fermentation process. Yields of yeast cell mass increased with time reaching a maximum at 168 hr. The inital pH of the fermentation medium was shifted to the acid side at the end of fermentation process.

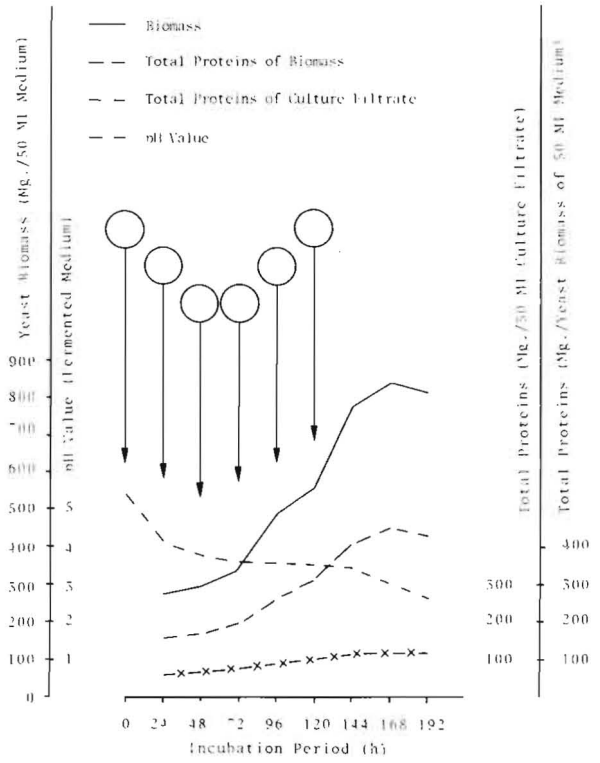

Fig. 4. Influence of diesel oil addition at different fermentation periods during growth of *Candida tropicalis*.

Increase of cell mass was also associated with an increase of total protein present in the yeast cell. Therefore, it is better to add diesel in small doses into the fermentation medium to avoid any harmful effects exerted on yeast growth by high diesel oil concentration.

Proteins are very essential nutrients for human beings, and the reports published by the FAO that the increase of population on this globe will practically double by the year 2000. The corresponding increase in consumption of proteins will amount to 100 million tons of additional proteins per year. Therefore, there is no hope that classical agricultural means adequate to cope with the potential protein shortage. Developing countries and territories which are in climatically difficult areas are totally dependent on the commercialization of foodstuffs.

Production of microbial protein represents a variable alternative. Therefore, man is now turning to the large scale production of microbial biomass through the production of SCP. SCP could be produced from hydrocarbons, petroleum derivatives (methanol, ethanol, and methane), carbohydrates (starch and cellulosic

wastes) and sugars (molasses, whey, sulphite liquor produced from wood pulping industries).

In Saudi Arabia, the main local materials are petroleum products and petroleum derivatives. Saudi Arabia Basic Industries Corporation (SABIC) will manufacture different petrochemical products. All of these projects will be built in the new industrial city of Al-Jubail on the East coast of Saudi Arabia. The Saudi Methanol Company will produce methanol commercially in 1984. Saudi Arabia is undergoing a rapid development and there is need for Single-Cell Protein to be produced locally to substitute other imported animal feeds. If these SCP products are useful for animal and human nutrition, this project will fit nicely in the Kingdom's industrialization plans, and would utilize locally produced products for food production.

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قدرات فطـــر الخمـيرة على إنتـاج بروتينـات الخلية الواحدة

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تم عزل خمائـــر محليـــة نشطـة من الهـواء والـتر بـة ورواسب المنتجـات النفطيـة. وبـاستـزراع ٦٧ عزلـة خميرة فى أوساط غذائية محتوية على زيت الديزل كمصدر وحيد للكربون لنمو الخمائر، تم اختيار أكفأ هذه العزلات إنتاجا لبروتينات الخلية الواحدة. وبيَّنت الدراسة أن أنشط كائنين من الخميرة هما:

كانديدا تروبيكالس (Candida tropicalis)

ياروبا ليبوليتكا (Yarrowia lipolytica)

وعند دراسة التغيرات الكيميائية الحيوية الحادثة أثناء التخمـر لإنتـاج بروتينـات الخليـة الـواحـدة بفطـر *كانـديدا تروبيكـــالس* اتسمت هذه التغـيرات بانخفـــاض فى الأس الهيـــدروجينى، وهـذا الانخفـاض يُمكن أن يُعـزى سببـه لتكـوين بعض الأحمـاض العضـويـة وغير العضوية فى وسط التخمـــر، كما أن كتلة خلايـــا الخميرة ازدادت بزيـادة فترة التخمـــر حتى بلغت أقصـاهـا عنـد ١٦٨ ساعـة. وأوضـح البحث أن التركيزات العالية من زيت الديزل لها تأثير مثبط على نمـو الخميرة، بينما أعطت التركيزات المنخفضة لزيت

الـديـزل فى حدود ٤٠ - ٦٠ سم ٣/لتر إنتـاجـاً جيـداً من بروتينـات الخليـة. ولقد ازداد إنتاج بروتينات الخلية الواحدة عند إضافة زيت الديزل على فترات متقطعة أثناء عملية التخمر.