Arab Gulf J. scient. Res. **3** (2), pp. 753-763 (1985)

Utilization of Different Petroleum Products in Production of Single-Cell Protein by *Candida lipolytica* YB-423

Abou-Zeid A. Abou-Zeid*, A.I. El-Diwany, M.A. Farid and H.M. Shaker

Natural Product Laboratory, National Research Centre Dokki, Cairo, Egypt

ABSTRACT. The results obtained revealed that diesel oil and *n*-hexadecane were the best carbon sources for the formation of SCP. Different types of petroleum products (diesel oil, kerosene and gasoline) from 'Morgan' and 'Shoeb Ali' wells were used for the formation of SCP by *C. lipolytica* YB-423. The protein content of 'Morgan' diesel oil SCP was about 42.5%. The suitable concentration of 'Morgan' diesel oil fraction No. 6 was about 14-18 ml/100 ml medium. Comparative studies of the protein content, profile of amino acids and some elements present in different SCP produced by *C. lipolytica* YB-423 grown on malt medium, and *Saccharomyces cerevisiae* grown on molasses medium were carried out.

Yeasts as well as other microorganisms have been grown on numerous substrates including waste materials such as blackstrap molasses, spent sulphite liquors and wood hydrolysate for formation of single-cell proteins to be utilized as animal feed and human foods.

In developed and oil producing countries, work has been conducted to use petroleum hydrocarbons for the production of single-cell proteins. Brown *et al.* (1969) reported that petroleum is an extremely complex mixture of hydrocarbons. Zobell (1950), Foster (1962), Mckenna and Kallio (1965), Van Der Linden and Thijsse (1965), Van Eyk and Bartles (1968) studied the metabolic pathways for the degradation of petroleum. They showed that the biodegradation of *n*-alkanes normally proceeds by a monoterminal attack, usually a primary alcohol is formed, followed by an aldehyde, and then a monocarboxylic acid. Ahearn *et al.* (1971)

* *Present address*: Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

examined yeasts that can utilize hydrocarbons, and have isolated different organisms: *Candida*, *Rhodosporidium*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces*, and *Trichosporon* which are able to utilize hydrocarbons. Simek *et al.* (1973) stated that the growth rate and protein content of yeast was affected by the ratio of nitrogen to phosphorus. Walker and Colwell (1976a and b) examined susceptibilities of hydrycarbons to microbial degradation, and noted that less biodegradation was obtained in a heavy fuel oil than in light fuel oil, and also less biodegradation occurred in heavy Kuwait crude oil than in a light south Louisiana crude oil.

The present paper deals with biosynthetic formation of single-cell protein using different Egyptian petroleum products and investigation of chemical components present in petroleum single-cell proteins, and comparing it with other types of single-cell protein produced by fermentation.

Material and Methods

Maintenance of the Experimental Organism

Candida lipolytica YB-423 was maintained on the following ingredients (g/litre): malt extract, 3.0; yeast extract, 3.0; peptone, 5.0; glucose, 10.0; agar, 25.0. The ingredients were mixed thoroughly and poured into test tubes (20 × 150 mm), each contained 10 ml medium. The tubes were plugged with cotton and sterilized at 1.2 atmospheric pressure for 20 min. The tubes were inoculated with *Candida lipolytica* YB-423 under aseptic conditions. The inoculated tubes were incubated at 30°C to obtain luxuriant growth. The slants were kept at 5°C in a refrigerator.

The same ingredients of the above medium without agar were prepared, mixed thoroughly, and the pH was adjusted to 5.5. The medium was portioned into Erlenmeyer flasks (capacity, 250 ml), each contained 50 ml. The flasks were plugged with cotton, sterilized at 1.2 atmospheric pressure for 20 min. When the flasks attained room temperature, they were inoculated under aseptic conditions with *Candida lipolytica* YB-423 grown in slants (one slant/50 ml medium). The inoculated flasks were inserted on a rotary shaker (200 rpm) at 30°C for 48 hr. At the end of fermentation period, the grown experimental organism was used as inoculum for fermentation process.

Basal Fermentation Medium

The basal fermentation medium contained the following ingredients (g/litre): $(NH_4)_2\ SO_4$, 5.0; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $MnSO_4.4H_2O$, 0.05; $FeSO_4.\ 7H_2O$, 0.005; and distilled water 1000 ml. The ingredients were mixed thoroughly, and the initial pH value was adjusted to 5.5. The medium was portioned into Erlenmeyer flasks (capacity 250 ml), each contained 50 ml medium. The flasks were plugged with cotton and sterilized at 1.2 atmospheric pressure for 20 min. The petroleum product or pure hydrocarbon was also sterilized at 121°C

for 10 min. There is no loss of volatile hydrocarbon materials during the sterilization process. When the flasks attained room temperature, sterile petroleum product or pure hydrocarbon was incorporated under aseptic conditions into the flasks. The percentage of petroleum product or hydrocarbon was 2.0 (v/v) in the fermentation medium. Each flask was inoculated with 0.5 ml of the previously prepared medium. The percentage of inoculum was 1.0. The inoculated flasks were inserted on a rotary shaker (200 rpm) at 30°C for seven days. At the end of incubation period, the final pH value, total dry weight of the experimental organism, total nitrogen and total crude protein present in biomasses were determined.

Source of Petroleum Products

Three petroleum products relating to 'Morgan' and 'Shoeb Ali' crude oil were obtained from El-Nasr Company for Oil Refinement (Suez, Egypt). The three products were gasoline, kerosene and diesel oil. *n*-Hexadecane was also obtained from B.D.H. Company (England).

Dry Biomass

At the end of the fermentation process, final pH value of the fermented medium was determined. The fermentation broth was centrifuged (6000 rpm) for 15 min. The precipitated microbial cell biomass was removed. The residual petroleum fraction was removed smoothly by suction; while the supernatant liquor, which contained an emulsified layer, was treated with *n*-hexane to dissolve the remaining residual petroleum products. Then, the supernatant liquor was centrifuged to precipitate the yeast cells which were adsorbed on to the hydrocarbon phase. The cells were collected and dried at 95-105°C until a constant weight was obtained.

Different Types of SCP

Different types of SCP were obtained for comparison. Single-Cell Protein produced by *Saccharomyces cerevisiae* grown on molasses medium (Egyptian Sugar and Distillation Company), and also SCP produced by *Saccharomyces carlsbergaunses* grown on malt medium (Al-Ahram Beer Company) were obtained to investigate their chemical constituents which compared with SCP produced by *C. lipolytica* YB-423 grown on medium containing petroleum product.

Total Nitrogen

Estimation of total nitrogen was carried out by the semi-Kjeldahl method (Miller and Houghton 1945, and Hawk *et al.* 1947). Protein has been calculated by multiplying total nitrogen by 6.25.

Amino Acids

Hydrolysis of crude protein was carried out according to the method of Block *et al.* (1958), while the paper chromatographic separation and determination of amino acids were carried out according the method of Whitehead (1964).

Elements

Phosphorus was determined by the method of Donal *et al.* (1956), and copper was obtained by the method of Morrison and Paige (1946); while iron was achieved by the technique of Mellon (1947).

Results and Discussion

Different Types of Egyptian Petroleum Products

The results obtained (Table 1) show that the conversion efficiency of the hydrocarbons to cell biomass was variable. The 'Morgan' diesel oil was the best petroleum one, suitable for formation of SCP by *C. lipolytica* YB-423. SCP obtained from 'Morgan' diesel oil was also rich in its protein content, while 'Shoeb Ali' SCP was less in protein content. The final pH values of fermented media were shifted into acid side. The pH decrease was due to the metabolism of ammonium (NH_4^+ ions), since that each time an ammonia ion was used, it left behind a hydrogen ion, and hence, the pH fell. Since ammonium ions were used for protein synthesis, and the increase to cell concentration probably reflected increased ammonium ion

Table 1. Role of different Egyptian petroleum products on production of SCP by *C. lipolytica* YB-423.

Type of petroleum fraction	Final pH value*	Dry biomass (g/100 ml)	Total protein (%)
n-hexadecane (B.D.H.)	3.7	0.9	40.2
'Morgan'			
Diesel oil	3.3	1.1	42.5
Kerosene	4.3	0.6	30.0
Gasoline	4.9	0.4	18.6
'Shoeb Ali'			
Diesel oil	3.4	0.1	39.3
Kerosene	4.4	0.5	26.8
Gasoline	5.0	0.4	16.1

* The initial pH value of fermentation medium was adjusted to 5.5.

utilization, and hence, a fall in pH. Biodegradation of *n*-hexadecane and diesel oil were more easily for the experimental organism, and consequently the formation of SCP. Protein contents of the different SCP-s were different, and high protein contents were presented in case of *n*-hexadecane, and diesel oil, while low protein content was obtained with kerosene and this may be due to the presence of low *n*-hydrocarbon content, and other protein inhibitors. Suitabilities of diesel oil as the sole carbon source for formation of SCP is mainly due to the presence of high percentage of *n*-hydrocarbons. *n*-Hydrocarbons were oxidised by oxygenases into acetaldehydes, and then into fatty acids. Fatty acids were converted into acetoacetic acid, and furthermore into 'active' acetate. Both 'Morgan' and 'Shoeb Ali' kerosene and gasoline products were less suitable for production of SCP. Therefore, diesel oil was selected for the further experiments.

Effect of Different Diesel Oil Fractions

'Morgan' diesel oil was fractionated into seven fractions by distillation at different temperature ranged from 210 to 330°C. These different fractions were utilized separately by *C. lipolytica* YB-423 as the sole carbon source and consequently formation of SCP. The results obtained (Table 2) show that the different 'Morgan' diesel oil fractions exhibited variable responses to formation of SCP by *C. lipolytica* YB-423. Different yields of dry biomass were obtained. Heavy fractions were more better than light ones in formation of SCP 'Morgan' diesel oil fraction No. 5 and 6 gave high titres of SCP. Therefore, fraction No 6 was conveniently selected as substrate for fermentative formation of SCP by *C. lipolytica*.

Table 2. Effect of different 'Morgan' diesel oil fractions on formation of SCP by *C. lipolytica* YB-423.

Fraction No.	Temperature (°C)	Final pH value*	Dry biomass (g/100 ml)	Total protein (%)
1	210-250	4.3	0.0	29.6
2	250-270	4.1	0.2	29.8
3	270-285	4.1	0.5	34.1
4	285-300	4.1	1.0	38.5
5	300-315	4.1	1.2	40.3
6	315-330	4.0	1.2	41.9
7	330	4.1	0.9	40.2
'Morgan' Diesel oil		3.5	1.3	42.5

* The initial pH value of the fermentation medium was adjusted to 5.5.

Concentrations of 'Morgan' Diesel Oil Fraction (315-330°C)

Different concentrations of 'Morgan' diesel oil were utilized as the sole carbon source for production of SCP. The results obtained (Table 3) show that the yield of SCP was increased with the increase of diesel oil fraction (315-330°C), reaching its maximum at 14.0-18.0 ml/100 ml medium. The fermentation medium contained limited nitrogen in assessing the final biomass concentrations. The initital pH value of fermentation medium was adjusted to 5.5, and at the end of fermentation process, the final pH value was changed. The final pH value was shifted to acid side, and this shift was increased with the increase of diesel oil fraction concentration. The shift of pH value to acid side might be due to the utilization of ammonia and formation of sulphuric acid, and also accumulation of organic acids, especially fatty acids and acidic amino acids. The protein content of diesel oil was higher than the protein contents of the different fractions. Accumulation of *n*-hydrocarbons in diesel oil fraction No. 6 (315-330°C) were also suitable carbon source for the production of SCP. *C. lipolytica* YB-423 could utilise these hydrocarbons easily towards biosynthesis of proteins. Tsugawa *et al.* (1969) stated that *Candida lipolytica* converts *n*-paraffins into α-ketoglutarate.

Constituents of Different Types of SCP Produced by Fermentation

Chemical constituents of different types of SCP produced by fermentation were investigated. Comparative compositions of the different types of SCP were carried out on SCP produced by *C. lipolytica* YB-423 grown on diesel oil medium, SCP produced by *Saccharomyces cerevisiae* (Egyptian Sugar and Distillation Com-

Table 3. Influence of different concentration of 'Morgan' diesel oil fraction No. 6 (315-330°C) on production of SCP by *C. lipolytica* YB-423.

Concentration (ml/100 ml)	Final pH value*	Dry biomass (g/100 ml)	Total protein (%)
2	4.2	0.3	35.3
6	3.6	0.8	35.8
10	3.2	0.9	37.1
14	3.1	1.4	41.9
18	3.0	1.4	42.1
24	3.0	1.4	40.9
30	2.9	0.8	35.9
'Morgan' Diesel oil	3.5	1.3	42.5

* The initial pH value of the fermentation medium was adjusted to 5.5.

pany) grown on molasses medium, and SCP produced by *Saccharomyces carlsbergaunses* (Al-Ahram Beer Company) grown on malt medium. The results obtained (Table 4) show that SCP produced by fermentation using diesel oil as the sole carbon source for the growth of *C. lipolytica* YB-423 contained more crude protein (42%), and crude fat (6.98) than the others.

Amino Acids Present in SCP

Amino acids present in the different SCP-s were revealed, and the results obtained (Table 5) show that the different types of SCP contained the following amino acids: cystine, cysteine, lysine, histidine, asparagine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, tyrosine, valine, methionine, tryptophan, phenylalanine, leucine, iso-leucine and proline. Concentrations of some amino acids present in SCP produced by diesel oil medium such as lysine, histidine, asparagine, serine, glycine, threonine, alanine, tyrosine, leucine, and isoleucine were higher than the other SCP-s. SCP produced from diesel oil contributes a good profile of amino acids and also good biological value.

Elements Present in SCP

The different types of SCP were subjected to wet oxidation (Pearson 1970), and determinations of phosphorus, copper, iron and zinc were carried out. The results obtained (Table 6) show that the different types of SCP contain variable amounts of phosphorus and small amounts of the elements are acceptable in the different types of single-cell protein.

Acknowledgement

The authors thank Dr. H.M. Salem, Professor of Biochemistry, Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt, for his interest and valuable discussion.

Table 4. Chemical constituents of different types of SCP produced by fermentation using different organisms and substrates.

Constituents (%)	Different types of SCP		
	Organism: *C. lipolytica* YB-423 Medium: (Diesel oil)	***S.* carlsbergaunses* (Malt**	***S.* cerevisiae* (Molasses)**
Crude protein	42.0	35.8	40.0
Crude fat	6.9	5.5	4.8
Crude ash	10.8	7.8	8.8

* ***Saccharomyces***.

Table 5. Amino acids present in different types of SCP produced by fermentation using different organisms and substrates.

Amino acids (μg/mg dry biomass)	Different types of SCP			Fish meal
	Organism: *C. lipolytica* YB-423 Medium: (Diesel oil)	*S.*carls-bergaunses* (Malt)	*S.* cere-visiae* (Molasses)	
Cystine & cysteine	8.15	5.71	5.71	10.37
Lysine & histidine	10.81	8.14	10.00	6.80
Asparagine	7.58	6.87	8.12	7.45
Aspartic acid	1.89	2.14	6.78	4.23
Serine & glycine	7.82	6.44	5.96	8.20
Glutamic acid	4.11	4.24	6.36	5.12
Threonine	26.11	25.71	25.71	20.41
Alanine	29.94	27.09	27.09	25.23
Tyrosine	11.89	20.31	11.97	11.75
Valine & methionine & tryptophan	6.94	7.53	6.84	5.30
Phenylalanine	7.14	20.00	8.66	2.05
Leucine & isoleucine	22.85	14.44	22.00	6.12
Proline	trace	trace	trace	trace

* *Saccharomyces*.

Table 6. Some elements present in different types of SCP produced by fermentation using different organisms and substrates.

Elements (μg/mg dry biomass)	Different types of SCP		
	Organism: *C. lipolytica* YB-423 Medium: (Diesel oil)	*S.* carls-bergaunses* (Malt)	*S.* cerevisiae* (Molasses)
Phosphorus	30666	21999	24666
Copper	10	77	11
Iron	152	204	275
Zinc	42	48	101

* *Saccharomyces*.

References

Ahearn, D.G., Meyers, S.P. and **Standard, P.G.** (1971) The role of yeasts in the decomposition of oil in marine environment, *Dev. Ind. Microbiol.* **12**: 126-134.

Block, R.J., Durrum, E.L. and **Zweig, G.** (1958) *A Manual of Paper Chromatography and Paper Electrophoresis*, 2*nd*. Ed., Academic Press. Inc. Publishers, W.D.

Brown, D.W., Romos, L.S., Fiedman, A.J. and **Macleod, W.D.** (1969) Analysis of trace levels of petroleum hydrocarbons in marine sediments using a solvent-slurry extraction procedure, *In*: *Trace Organic Analysis*: a new frontier in analytical chemistry. Special publication no. 519. National Bureau of Standards, Washington, pp. 161-167.

Donald, R., Schwer, E.W. and **Wilson, H.N.** (1956) Recent advances in the determination of phosphate in fertilizers, *J. Sci. Food. Agric.* **7**: 677-690.

Foster, J.W. (1962) Hydrocarbons as substrates for microorganisms, *J. Microbiol. Serol.* **28**: 241-249.

Hawk, F.P., Oser, L. and **Summerson, W.H.** (1947) *Micro-Kjeldahl method. Practical Chemistry*, 12*th*. edition, J. and A. Churchill Ltd., London, p. 1323.

Mckenna, E.J. and **Kallio, R.E.** (1965) Microbial metabolism of isoprenoid alkanes, *Proc. Natl. Acad. Sci. USA*, **68**: 1552-1554.

Mellon, M.G. (1947) Colorimetric determination of iron with nitrose R-salt, *Anal. Chem.* **19**: 1014-1016.

Miller, L. and **Houghton, J.A.** (1945) Modification of Kjeldahl, *J. Biol. Chem.* **159**: 373-377.

Morrison, S.L. and **Paige, H.L.** (1946) Modified all-dithizone method for the determination of traces of copper, *Ind. Eng. Chem.* **18**: 211-213.

Pearson, D. (1970) *The Chemical Analysis of Food*, 6*th*. ed., J. and A. Churchill, London, pp. 24-27.

Simek, F., Eva, S.M. and **Borbiro, B.G.** (1973) Role of nitrogen and phosphorus in protein synthesis by *Candida guilliermondii* cultivated on *n*-alkanes, *Biotechnol. Bioeng.* **4**: 155-60.

Tsugawa, R., Nakase, T., Kobayashi, T.K. and **Okumura, S.** (1969) Biological oxidation of *n*-hydrocarbons by *C. lipolytica*. *Agr. Biol. Chem.* **33**: 629-634.

Van der Linden, A.C. and **Thijsse, G.J.** (1965) The mechanisms of microbial oxidations of petroleum hydrocarbons, *Adv. Enzymol.* **27**: 469-546.

Van Eyk, J. and **Bartles, T.J.** (1968) Parrafin oxidation in *Pseudomonas aeruginosa*. I. Induction of parrafin oxidation, *J. Bacteriol.* **96**: 706-712.

Walker, J.D. and **Coldwell, R.R.** (1976a) Long-chain *n*-alkanes occurring during microbial degradation of petroleum, *Cand. J. Microbiol.* **22**: 886-891.

Walker, J.D. and **Colwell, R.R.** (1976b) Measuring the potential activity of hydrocarbon-degrading bacteria, *Appl. environ. Microbiol.* **31**: 189-197.

Whitehead, R.G. (1964) Rapid determination of some plasma amino acids in subclimical Kwashiorkor, *Lancet* **1**: 250-254.

Zobell, C.E. (1950) Assimilation of hydrocarbons by microorganisms, *Adv. Enzymol.* **10**: 443-486.

(Received 02/11/1983;
in revised form 09/03/1985)

الاستفادة من المنتجات النفطية فى إنتاج بروتينات الخلية الواحدة بفطر *كانديدا ليبوليتكا* (*Candida lipolytica*)

أبوزيد على أبوزيد، أحمد ابرهيم الديواني، محمدعبدالفتاح فريد و حسام محمد شاكر

معامل المنتجات الطبيعية - المركز القومي للبحوث - الدقى - جيزة - مصر

تمت الاستفادة من *n*-hexadecane وبعض المنتجات النفطية الأخرى كمصادر كربونية فى إنتاج بروتينات الخلية الواحدة بفطر *كانديدا ليبوليتكا*. أظهرت نتائج البحث أن زيت الديزل و *n*-hexadecane أفضل مصادر كربونية للمنتجات النفطية. ولقد استخدم فى البحث منتجات نفطية مختلفة من إنتاج آبار «مرجان» و «شعيب على» مثل زيت الديزل والكير وسين والجازولين، وتبين أن منتجات بئر «مرجان» مناسبة كمصادر كربونية فى التكوين الحيوى لبروتينات الخلية الواحدة بفطر *كانديدا ليبوليتكا*، وكان أفضل تركيز لقطفة زيت الديزل رقم ٦ فى تكوين هذه البروتينات ما بين ١٤ إلى ١٨ سم^{٣}/١٠٠ سم^{٣} لوسط التخمر.

وفى هذا البحث تمت دراسة مقارنة لتحليل المحتوى البروتينى والأحماض الأمينية وبعض العناصر الموجودة فى أنواع مختلفة من بروتينات الخلية الواحدة والناتجة من تخمر أوساط بيئية مختلفة تحتوى على زيت الديزل والمولاس

والشعير، وتبين من نتائج التحاليل أن بروتينات الخلية الواحدة الناتجة من زيت الديزل تتميز بوجود نسبة عالية من البروتين وبعض الأحماض الأمينية تفوق مثيلاتها من بروتينات الخلية الواحدة الأخرى. كما بينت الدراسة وجود بعض العناصر بتركيزات منخفضة فى الأنواع المختلفة لهذه البروتينات.