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Evaluation of nutritive values of SCP (*Chlorella vulgaris*) propagated in palm oil mill effluent sludge

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Chlorella vulgaris, isolated from scums of effluent sludge produced by palm oil mill-refinery, has already been reported to overcome the significant pollution load of this agro-based industry in Malaysia and it has considerably met the requirements of the established environmental standards. From the viewpoint of economic viability of this SCP, *Chlorella vulgaris*, by-product was found to be fairly high in protein; 43.9%, phosphate; 6,000 ppm, K; 19,600 ppm, Na; 56,000 ppm, Ca; 9,600 ppm, Mg; 13,500 ppm and Fe; 450 ppm, contents. Fatty acids of total lipids, 2.83%, are also comparable to those reported for unicellular algae, *Dicrateria inornata* and *Isochrysis galbana*. The amino acids composition are also comparable to *Chlorella vulgaris* F & M cultured in the liquid phase of pig slurry for recycling of nutrients. Steroidal component was 0.019%, and it was composed of three unknowns, viz. A, B & C, of which unknown C is the predominant species while cholesterol, ergosterol and β-sitosterol prevailed at 1.722, 123.45 and 111.64 ngs. mg^{-1} crude extract, respectively.

Key Index Words: Chlorella vulgaris; *nutritive evaluation*; *palm oil mill refinery effluent sludge.*

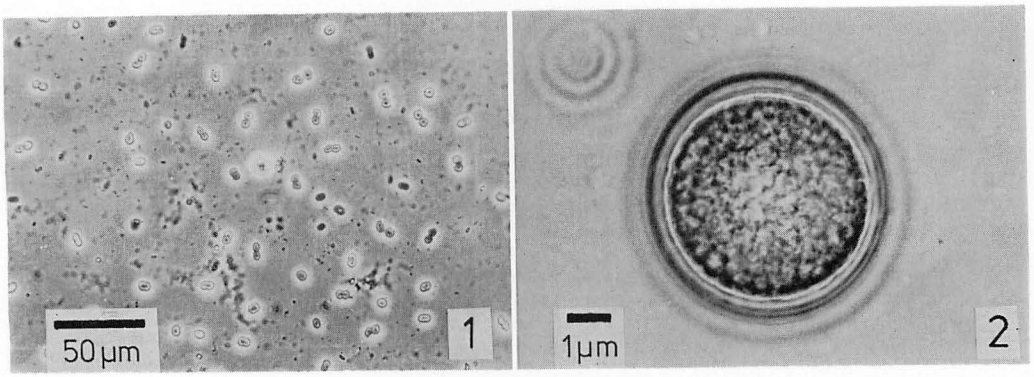
"Palm Oil", the Malaysian golden crop is expected to increase its annual production of 3 million tonnes to four million tonnes by 1985, subsequently surpassing the production of natural rubber. On the contrary, as of last year approximately 7.5 million tonnes of effluent valued at $ 56 millions was indiscriminately discarded into the aquatic ecosystem creating a facet of agro-waste pollution problems. If properly managed, these could be reprocessed into fertilizer, animal feed, biogas or single-cell proteins.

The production of biogas, based on the above mentioned figures, is estimated to probably meet 7% of the national electricity output at a cost of 14 cents per unit when compared to the current rate of 17 cents. Conversion into fodder, on the other hand, could produce 100,000 tons of beef minimizing $ 80 millions per year in importing beef. From the viewpoint of pollution control, under the Environmental Quality (Sewage and Industrial Effluent) Regulation of 1979, factories are supposed to have proper treatment plants, but unfortunately many do not adhere to this rule and have ultimately destroyed the biota of most local rivers.

The biological approach of propagating single cell proteins, *Chlorella vulgaris*, in palm oil refinery mill sludge as a feasible means of lowering the B. O. D. load from 1,080 ppm to 40 ppm had been reported earlier by the author (SIVALINGAM 1980a & b, SIVALINGAM *et al.* 1979). This manuscript endeavours to illustrate the nutritive values of the harvested *Chlorella vulgaris* from the stance of employing them for human as well as animal consumption.

Materials and Methods

Isolated *Chlorella vulgaris* cells (Figs. 1,



Figs. 1-2. Propagated SCP (*Chlorella vulgaris*).

2) were analyzed for their general mineral contents, protein, lipid and steroidal components.

Minerals were analyzed employing a Varian Techtron AA120 atomic absorption spectrophotometer while protein content by the routine Kjeldahl method. The amino acids components of the protein moiety were detected using a JEOL-JLC-5AH Automatic Amino Acid Analyzer. Lipids were extracted with the Soxhlet apparatus and its fatty acid components subjected to GLC analysis on methylation with boron trifluoride in methanol. The steroidal components extracted according to the methods of IKEKAWA *et al.* (1968) and ISABELLE CHARDON-LORIANX *et al.* (1976) were determined by direct injection into a Shimadzu gas chromatograph GC-6A, attached with a hydrogen flame ionization detector.

Results

Table 1 indicates the biochemical data of *Chlorella vulgaris*. Evidently, the protein content is high with phosphate, K, Ca, Mg and Fe contents falling in the same category. Without a shadow of doubt, this reflects the possibility of utilizing the cultured unicellular algae as human food and/or animal feeds. The amino acid composition of the crude proteins was, as indicated in Table 2, comparable to those obtained for *Chlorella vulgaris* F & M cultured in the liquid phase of pig slurry and recycled for animal feed, indirectly reflecting the suitability of the present product.

Table 1 Biochemical Analysis of *Chlorella vulgaris* Cultured in palm Oil Mill Sludge

Parameters	Values
Crude protein (%)	43.9
Crude lipid (%)	2.83
Ash (%)	8.33
P (ppm)	6,000
K (ppm)	19,400
Na (ppm)	56,000
Ca (ppm)	9,600
Mg (ppm)	13,300
Fe (ppm)	450
Cd (ppm)	1.9
Co (ppm)	BDL*
Cr (ppm)	BDL
Cu (ppm)	7.0
Ni (ppm)	BDL
Pb (ppm)	BDL
Zn (ppm)	43

* BDL; Below detectable level.

The crude lipid's fatty acid components are given in Table 3. When compared with those of unicellular algae, *Dicrateria inornata* and *Isochrysis galbana* (WATANABE and ACKMANN 1974) they appear to be related but only that the palm oil mill sludge cultured *Chlorella vulgaris* had the saturated fatty acid 14:0 as its largest component. Since *Dicrateria inornata* and *Isochrysis galbana* are extremely nutritous as supplement of food to culture oysters, obviously *Chlorella vulgaris* does not seem to be deficient in any essential fatty acid moiety from the viewpoint

Table 2. Amino acids composition of *Chlorella vulgaris* grown in palm oil mill sludge and its comparison with those (*Chlorella vulgaris* F & N) grown in the liquid phase of pig slurry*.

Essential amino acids	*Chlorella vulgaris* present exp. (%)	*Chlorella vulgaris* F & N (%)	Other amino acids	*Chlorella vulgaris* present exp. (%)	*Chlorella vulgaris* F & N (%)
Arg	5.13	5.49	Ala	8.03	10.07
Cys	0.35	0.31	Asp	9.09	8.24
Gly	8.75	6.82	Glu	9.90	15.16
His	0.89	2.44	Pro	4.93	7.94
Ileu	5.35	3.66	Ser	5.32	4.48
Leu	8.79	7.94	NH_3	9.87	—
Lys	3.46	10.89			
Met	2.20	0.61			
Phe	1.55	3.46			
Thr	5.61	4.27			
Try	1.64	1.42			
Tyr	3.26	2.95			
Val	5.86	3.87			

* Cited from: Kerry Garett, M., John J. Strain and Marion M. B. Allen (1976). Composition of the product of algal culture in the liquid phase of animal slurry. J. Sci. Fd. Agric. 27: 603-611.

Table 3. Fatty acids components of the total lipids of *Chlorella vulgaris* cultured in palm oil mill sludge as compared to that of *Dicrateria inornata** and *Isochrysis galbana**.

Fatty acids	Percent		
	Chlorella vulgaris	*Dicrateria inornata*	*Isochrysis galbana*
12:0	1.5	—	—
?	0.2	—	—
?	0.3	—	—
14:0	40.5	11.9	10.6
14:1	3.2	—	—
15:0	1.5	3.7	1.3
?	1.5	—	—
16:0	8.3	30.9	22.2
17:0	—	0.6	1.1
18:0	0.5	2.4	2.3
16:1W9	—	1.5	1.4
16:1W7	30.3**	10.2	14.3
?	0.3	—	—
18:1W9	3.0**	10.1	3.8
18:1W7	—	4.5	9.3
20:1	1.1	—	—
20:1W11	—	NSA***	0.3
20:1W9	—	3.5	TRA
20:1W7	—	0.4	0.9
22:1W13 & 11	—	1.2	TRA
22:1W9	—	0.5	NSA
22:1W7	—	0.1	NSA
22:4W9	0.1	—	—
16:3W3	—	5.8	0.4
18:2W6	1.6	2.5	2.3
18:3W6	0.3	TRA****	0.2
18:3W3	0.3	3.1	0.4
18:4W3	0.2	0.3	8.0
20:2W9	0.3	—	—
20:3W9	0.1	—	—
20:4W6	—	0.2	0.1
20:5W3	—	0.5	7.2
22:6W3	—	TRA	4.3

* Cited from Watanabe and Ackman, 1974.
** Small amounts of other moncenes were included.
*** NSA: non-saponificable acids.

of fatty acid components.

Crude steroidal content was ca. 0.019% and the steroidal components are as indicated in the gas chromatogram of Fig. 3. These components separated are made-up of cholesterol ergosterol and β-sitosterol at 1.722, 123.45 and 111.64 ngs. mg^{-1} crude extract, respectively, along with three unknown classified as A, B and C. These unknown components are of great interest, especially the unknown C which appears to exist in the largest quantity, and should be examined to

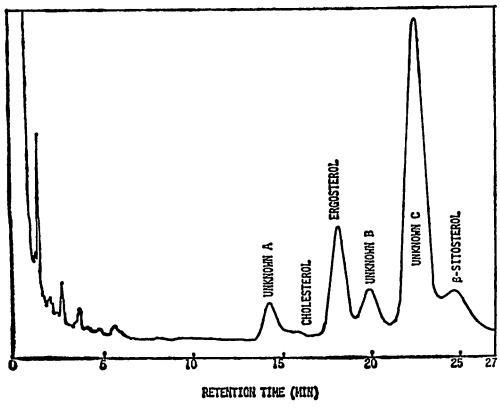


Fig. 3. Gaschromatogram of the steroidal components of *Chlorella vulgaris* propagated in palm oil mill effluent sludge.

clarify whether they possess any biological activity. It should be noted here that the cholesterol component being in an extremely low content favours its easier conversion as a dietary source.

Discussion

It has already been indicated that via SCP (=single cell protein) propagation the B.O.D load of palm oil mill refinery sludge can be lowered to as low as 50 ppm to meet the local water quality criteria. Recycling of *Chlorella vulgaris* for human/animal feed depends greatly on its food values. Owing to its high protein content, certain minerals, suitable fatty acid components and low cholesterol content, this aspect is very encouraging. Hence, if the plant was properly managed, this algal by-product could turn out to be a killer for the foreign exchange earners when exported to countries where the demand for such an algal source is great. Ultimately, this by-product could augment the earning of organizations involved in the palm oil industry while concurrently eliminating the headache of environmental pollution.

Further, if the unknown steroidal components are identified to be biologically active the value of this by-product would again be upgraded.

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P. M. シバリンガム： ヤシ油用製粉所からの排出汚物で培養した SCP 用 *Chlorella vulgaris* の栄養価

ヤシ油用製粉所から排出される汚物は，マレーシアにおいて環境汚染と絡んで問題となっている。筆者はこの点を解決すべく，この汚物より分離した *Chlorella vulgaris* をそれで培養することにより，浄化(既報)と培養したクロレラを動物もしくは人間の食べ物にしようとして実験を行った。このクロレラはタンパク質に富み (43.9 %)，構成アミノ酸種およびその量比も既に利用されているブタ排泄汚物で培養したクロレラに類似しており，無機塩（りん酸塩，K, Na, Ca, Mg, Fe など）の含有量も SCP の観からは十分であった。また，ステロール類を分析したところ，コレステロールは最も少なくエルゴステロールや β-シトステロールには比較的多く，このほか未同定の3成分 (A, B, C) があり，うち C は全成分中最高であった。(School of Biological Sciences, University of Sciences Malaysia, Minden, Pulau Pinang, Malasia)