Algal single cell protein production from sewage effluent with high salinity

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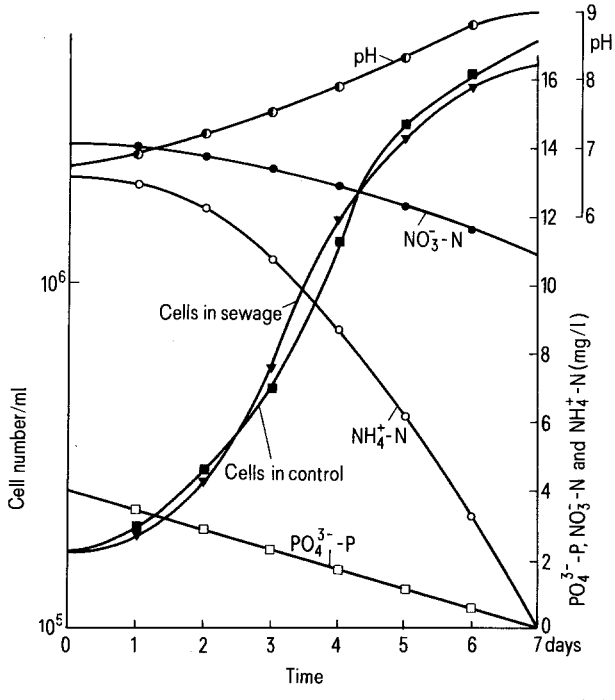
Summary. Laboratory studies indicate that the unicellular green alga *Chlorella salina* CU-1 could be cultivated in treated sewage effluent with high salinity. The high protein content (51% dry weight), and the relatively complete amino acid profile of the cells, suggest that this alga might be an ideal organism to be used for single cell protein production from high-salinity sewage.

Algae have been used extensively in stabilization ponds[1], in lagoons[2] and in tertiary treatment of domestic sewage[3] for removal of inorganic nitrogen and phosphorus from the wastewater due to their ability to utilize these inorganic nutrients. In addition, sewage-grown algae have been found to be source of low-cost algal protein which can be used as animal feed[4–6]. A majority of the studies on sewage-grown algae dealt with freshwater sewage effluent[7–10]. There is no published literature on the production of algal single cell protein in sewage effluents with high salinities. The domestic sewage effluent in Hong Kong is characterized by having a relatively high salinity since seawater is supplied for flushing systems[11]. As part of a study concerning the feasibility of producing algal biomass for animal feed by mass cultivation of unicellular green algae in sewage effluent in Hong Kong, we found that a locally isolated strain of *Chlorella salina* can serve such a purpose.

Materials and methods. Chlorella salina CU-1 was isolated from estuarine water from Tolo Harbour, New Territories, Hong Kong. The sewage effluent was obtained from the sewage treatment plant of The Chinese University of Hong Kong, Shatin, Hong Kong. Quantitative analysis of the inorganic N and P in the sewage effluent was carried out by standard methods[12–14]. The 'modified complete medium'[15] with added NaCl (1.5%) was used as the control medium. Cells of *C. salina* CU-1 were inoculated into duplicated cultures of both sewage effluent and the modified complete medium (initial inoculum: 3×10^5/ml) and were grown under 4000 lx light intensity with a 16/8 light-dark cycle at 25 °C with aeration. Growth, as measured by direct cell count, was monitored daily until the stationary phase was reached. The changes in pH and in levels of NH_4^+-N, NO_3^--N and PO_4^{3-}-P in the sewage effluent were recorded daily. When growth was completed, the cells were harvested by centrifugation and the cell pellets were dried in an oven at 110 °C for 1 h. Crude protein, fat, carbohydrate and fibre contents of the dried cells were determined by standard methods[16]. The ash content was determined after ignition at



Growth of *Chlorella salina* CU-1 in sewage effluent and in control medium. The change of pH and utilization of NH_4^+–N, NO_3^-–N and PO_4^{3-}–P by the algal cells in the sewage effluent are also shown.

Table 1. Chemical composition of *Chlorella salina* CU-1 cultivated in sewage effluent

Constituent	g/100 g/dry algae
Crude protein (N × 6.25)	51
Crude fat	17.82
Carbohydrate	11.25
Ash	17.5
Crude fibre	7.2
RNA	1.37
DNA	0.72
	mg/100 g/dry algae
Vitamin A	58.17
Niacin	4.7
Thiamine	1.83
Riboflavin	5.21
Vitamin C (ascorbic acid)	62.34

Table 2. Amino acid composition of *Chlorella saline* CU-1 cultivated in sewage effluent

Amino acid (g/16 g N)	*C. salina* CU-1	FAO patterns*
Alanine	4.9	
Arginine	8.9	
Aspartic acid	5.1	
Glutamic acid	4.3	
Glycine	3.2	
Histidine	2.0	
Isoleucine**	1.1	4.0
Leucine**	4.2	7.0
Lysine**	6.2	5.5
Methionine + cystine**	2.1	3.5
Phenylalanine + tyrosine**	5.0	6.0
Proline	1.0	
Serine	4.7	
Threonine**	3.4	4.0
Tryptophan**	1.9	1.0
Valine**	6.4	5.0
Total amino acids	64.4	

*Energy and protein requirements, Report for a joint FAO/WHO ad-hoc expert committee, No.*52*, 63 (1963). **Essential amino acids.

550 °C. Nucleic acid contents were determined according to the methods of Smillie and Krotkov[17]. In addition, the content of vitamin A, thiamine, riboflavin, niacin and vitamin C was also determined[16,18]. The amino acid composition of the algal protein was determined using an amino acid analyser (Beckman Model 120C).

Results. The salinity of the sewage effluent was found to be 15 ppt. The initial concentrations of inorganic N and P in the sewage effluent are shown in the figure. Growth of the cells in the sewage effluent was found to be comparable to that of cells grown in the modified complete medium. Apparently, cells of *C. salina* CU-1 preferred NH_4^+-N as the major nitrogen source, and NO_3^--N was only used by the cells to a much smaller extent. Both levels of NH_4^+-N and PO_4^{3-}-P in the sewage effluent dropped to zero on the 7th day of cultivation. It is conceivable that cells of *C. salina* CU-1 can be used for purification of wastewater having a high salinity due to their high efficiency in removing the inorganic nutrients.

Table 1 shows the chemical composition of the algal cells. The crude protein content of the cells was found to be 51%. It has been reported that sewage-grown algae or seawater-grown algae generally have a higher ash and a lower protein content[19]. However, the protein content of *C. salina* CU-1 was found to be higher than that of most other sewage-grown algae[5], and comparable to that of others[6]. The content of vitamin A, vitamin C and riboflavin in the cells was higher than that of most other sewage-grown algae, while that of niacin was lower than average[5]. The levels of the vitamins tested were in general found to be higher than those reported for *Scenedesmus acutus* grown in fertilizer with added molasses[20] and for *Chlorella-Scenedesmus* cultures grown in sewage effluent[5]. On the other hand, the low level of total nucleic acids (2.09%) of the cells provides an advantage in using the algal cells as possible supplementary animal feed, since high levels of nucleic acids might cause harmful effects on animals[21]. It is also conceivable that the low crude fibre content (7.2%) of the cells offers yet another advantage, in increasing the digestibility of the algal biomass when used as animal feed. Analysis of the protein of the algal cells revealed a rather complete amino acid profile and all the essential amino acids were present in the protein (table 2); this compares well with the FAO patterns. The total amount of amino acids (64.4 g/16 g N) is comparable to levels reported for other *Chlorella* species grown in artificial media[22,23]. Experiments on pilot-scale outdoor cultivation of *C. salina* CU-1 in sewage effluent are now in progress.

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A new host species for lactic dehydrogenase virus

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Summary. Lactic dehydrogenase virus has been found to replicate and maintain a chronic infection in the Asian mouse *Mus caroli* as it does in *Mus musculus.* However, the level of viraemia is lower and the increase in plasma lactate dehydrogenase activity very much less.

Lactic dehydrogenase virus[2,3] (LDV), a non-pathogenic virus, readily infects all strains of wild and laboratory mice *Mus musculus,* in which its infectivity has been tested. In these mice there is lifelong viraemia and raised plasma enzyme levels. The plasma lactate dehydrogenase (LDH) activity is 5-10 times the normal level by 72 h after infection and it is by this increase that the infection is most easily diagnosed. Infection has not been reported in any other species. Plagemann and his colleagues[4] injected rats and golden Syrian hamsters with LDV but there was no elevation of LDH activity in the plasma and virus infectivity could not be demonstrated in the plasma 1-2 weeks after virus injection. Notkins[5] reported that the injection of LDV into rats, hamsters, guinea-pigs or rabbits did not cause a rise in plasma LDH activity and attempts to demonstrate infectious virus in the plasma of these animals were unsuccessful. Deer mice, *Peromyscus maniculatus* have also been reported as insusceptible[6].

In an attempt to find another host species for the virus, dwarf hamsters *Phodopus sungorus* and the Asian mouse *Mus caroli* were used. Young adult animals were injected i.p. with a large dose of virus (10^7 ID_{50}) and blood samples