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DIVISION OF WATER TECHNOLOGY

MICROCYSTIS SCUMS FROM HARTBEESPOORT DAM AS A SOURCE OF FINE CHEMICALS

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EXECUTIVE SUMMARY

This report consists of two parts, firstly an extensive literature review covering the numerous aspects that influence micro-algal production, and, secondly, an evaluation of the potential of harvesting the algal scums from Hartbeespoort Dam. The literature review covers literature until the end of 1989 and evaluates environmental factors affecting productivity, the nutritional value of algae as a source of animal and human feed, toxicology and economic potential of algal products.

The second part reports on the chemical analyses of *Microcystis* scums from Hartbeespoort Dam. Data are given on the potential value of fine chemicals from the scum and cost factors. Although the algal material has a great potential value it is concluded that economic exploitation would not be readily feasible at present due to the seasonal variation of algal species and biomass in the dam. Another aspect mitigating against the commercial exploitation is the variability recorded in the concentration of the desirable fine chemicals.

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PART 1

LITERATURE REVIEW

MICROALGAE

RECENT DEVELOPMENTS IN PRODUCTION AND USE

INTRODUCTION

This literature survey focuses on the potential uses of algae. Most of the information in this review has been derived from open air mass cultivation experiments in numerous parts of the world. However, information should be applicable to algae developing in hypertrophic conditions such as Hartbeespoort Dam.

The two predominant characteristics of microalgae are the high efficiency by which they are able to convert solar energy into cellular biomass, and the high proportion of the biomass that is protein. In effect, the yields available via the mass cultivation of microalgae amount to between 20 to 50 times more protein in areal terms than peak soybean yields,[1] with productions achievable of 50 to 110 tonnes/ha/y[2], and potential maxima amounting to 500 tonnes/ha/y[3].

Another evident benefit of mass algal cultivation is that microalgae can utilize poor quality waters, such as brackish, saline and wastewaters that are unsuitable for use in agriculture, not only for the production of protein, but also in the upgrading of waters for secondary usage,[4-8] aquaculture,[9-10] or the production of fine chemicals and health foods.[11-15]

The consumption of microalgae by man in parts of the world dates back for many centuries.[16,17] During Cortez's expeditions into South America the Aztecs ate a cheese like material made from a 'slime' collected from Lake Texoco, near where the blue-green alga *Spirulina* is still growing and where it is commercially harvested today.[18,19] The same genus is also collected from Lake Chad in Africa, whilst the lake plum, *Nostoc* sp. which forms pea size round nodules, is eaten by inhabitants of Northern Thailand and the Peruvian Andes.[20]

The filamentous green algae *Oedogonium* sp. and *Spirogyra* sp. are used in Burma, Thailand, Vietnam and India and the pluriseriate green alga *Prasiola* sp. in China and Japan.[21]

Intensive cultivation of microalgae was initiated in the 1940's in Germany,[22] gaining encouragement from the Carnegie Institute of Washington during the 50's,[23] and has since been taken up in many countries, including Holland,[24] Japan,[25] Czechoslovakia,[26] Rumania,[27] Soviet Union,[28] France,[29] Belgium,[30] India,[31] Israel,[2] Italy,[32] Mexico,[33] Peru[34] South Africa,[35] Taiwan[36] United States,[2] Great Britain,[8] Bulgaria,[27] Poland[37] and Portugal.[38]

Certain microalgal species tend to dominate in mass-culture systems, regardless of which algae are used as inocula.[2] These include the 'weed' species of the genera *Chlorella, Micractinium* and *Scenedesmus* in freshwater systems and *Phaeodactylum* and *Skeletonema* in marine systems.[39] However, in 'clean culture' systems a single algal type can be maintained over extended periods, as, for example, *Scenedesmus obliquus*,[40] *Coelastrum probiscideum*[41,42] and *Spirulina platensis*.[43] For instance, *Coelastrum probiscideum* was

cultured continuously on a semi-technical scale in Germany for at least seven years, and *Stigeoclonium* has been cultivated for at least four years in secondary effluents in South Africa.[6,44] The use of extreme environmental conditions tend to select for specific algae. For example *Dunaliella* is produced in highly saline conditions,[45] and *Spirulina* in high bicarbonate environments.[33] High temperatures could select for thermotolerant algae, although mesophyllic algae such as *Scenedesmus acutus*, which have a laboratory temperature limit of 34 to 35 °C, can survive short-term heating to 42 °C.[40]

In natural or man-made lakes algal diversity decreases with increasing eutrophy and in hypertrophic systems complete domination by a single species can occur.[46] In smaller hypertrophic systems characterized by high productivity algal blooms often collapse and decompose,[46] but in larger systems such as Hartbeespoort Dam stable populations of *Microcystis* are known to persist for months,[47] thus making it possible to exploit the potential algal production in such systems for useful products.

FACTORS AFFECTING PRODUCTIVITY

Light

Environmental parameters, such as temperature, nutrients and ecological balances, play an influential role, particularly under non-ideal conditions, yet light is generally considered to be the primary yield determinant.[1,2,48-52] Adequate agitation and mixing therefore have a direct bearing on the degree to which the individual algal cells are subjected to light and dark conditions.

The light available to the algal cells may be described by the Beer Lambert expression[103]

$$I = I_o\, e^{-UXD}$$

in which I_o is the light intensity at the surface (μEinsteins/m^2/s). U the light extinction coefficient specific to the culture (cm^2/mg), X the biomass concentration (mg/ℓ) and D the depth below the surface (cm). In practical terms this implies that a surface light intensity of 1 900 μE/m^2/s (peak midday intensity) would be attenuated to the compensation point of approximately 20 μE m^2/s (where photosynthetic activity balances respiratory activity) in a depth of only 60 mm of a culture density of 400 mg/ℓ.[53]

The Beer Lambert law is really only applicable to dilute solutions illuminated by monochromatic light and possessing a small scattering component.[4] With filamentous algal cultures, or in the presence of extraneous particulate matter (bacteria, solids etc.) the availability of light to the algae can be significantly diminished. This accounts for calculated

photic zones ranging from only 20 mm at high algal concentrations in cattle feedlot effluent to 350 mm for low algal concentrations in clear media.[8,53-55] In practice increasing the culture density reduces the photosynthetic zone and thus the net specific growth rate of the culture. Conversely, reducing biomass concentration increases light penetration and overall growth rate.[52]

The optimal light intensity for most freshwater algae is of the order of 80 W/m^2 Photosynthetically Available Light (PAL) or between 2 000 - 5 400 lux.[3,56-64] In actual energy terms a quantum demand of 20 quanta per mol CO_2 assimilated is required.[65] This value can be influenced by species, exposure time and cellular density. The optimal algal density in open systems is in the range of 35-80 g/m^2,[2, 52,43,66] although indoor, illuminated closed systems may be as high as 2 000 g/m^2.[3]

pH

The pH of the median influences the algal metabolism directly through enzymatic control and indirectly through the availability of nutrients, minerals and trace elements.[67-69] Under alkaline conditions, (pH >8,6) the solubility of phosphate decreases as it combines as salts of calcium and magnesium, whilst ammonia may be lost through volatilization to the atmosphere and conversion from the NH_4^+-N form into the more toxic NH_3 form.[6,70] Abelovich and Azov[71] considered the upper pH limit for undisturbed phoynthesis in the presence of ammonia to be 7,9, with a concentration of 2 mM/ℓ. Growth limitation can be correlated to low CO_2 at high pH, or to high CO_2 at low pH, which can inhibit cell division and directly influence the production of biomass and carotenoids.[2,11]

In uncontrolled open air systems, the pH may rise as high as 11[6,37,72,73] as photosynthesis consumes the available CO_2 causing the algae to enter potentially limiting conditions. Under these conditions CO_2 should be supplemented to control the pH to more conducive levels for growth. In thick scums of *Microcystis* on Hartbeespoort Dam pH values in excess of 10 have been measured.[74]

Temperature

In general, the temperature dependence of algal growth can be described by the Arrhenius equation where it displays an exponential increase of yield until the optimum is reached, above which yield decreases rapidly.[75-78] Algal species have developed to occupy the whole temperature sphere, from cryophilic (cold loving), mesophyllic, which includes all

the candidates for commercial aquaculture, to thermophilic (heat loving) with optima over 40 °C . For specific species, and depending on its thermal response characteristics, only a portion of the Arrhenius relationship is valid.[52,35]

The influence of temperature is interrelated to other environmental factors, particularly light, although the direct relationship can be variable and rapid adaptations to changing conditions influencing the development of low and high temperature tolerant strains.[76-83]

The saturating light intensity will usually increase proportional to temperature, although to low intensity, increasing temperature decreases photosynthetic rate, whilst at high intensity, increasing temperature increases photosynthetic rate.[52] Similarly, at low temperatures, increased irradiance decreases algal growth, whilst at high temperatures irradiance enhances growth.[83,84] Photoinhibition is also temperature dependent, accounting for up to 67% of growth limitation at 30 °C, compared to 4,3% at 0 °C . Dark respiration (with a Q_{10} of 2) also depends upon the temperature.[52]

Nitrogen

Although, there are exceptions due to physiological and environmental variations, growth as well as the phenomenon of luxury uptake,[85,86] algal nitrogen (N) content is generally taken to be 9 to 12% by weight with phosphorus (P) 0,9 to 1,5% and carbon 35 to 60%.[22]

The ability to use any of nitrate, nitrite and ammonia appears to be common, as does the limited uptake of organic forms.[22, 87-90] When taken in the oxidized form, the nitrogen must be reduced before it can be incorporated into organic molecules. This requires additional energy and accounts for the preferential utilization of ammonia over other nitrogen forms.[6,91,92] Nitrate may also become toxic at much lower concentrations than other organic forms.[93] Among the prokaryotes, many of the cyanobacteria are also able to directly fix gaseous nitrogen into assimilatible forms.

There have been some conflicting reports as to the influence of growth rate on algal nitrogen content, with both increases and decreases of nitrogen uptake rate observed in response to algal growth rate increases. It is probable that factors in addition to species, enzymatic pathways and external environmental conditions contribute to the conflict, as well as the algal N and P content itself.[94-101]

The K^2 or half saturation concentration for nitrogen range from 0,01 - 50 µM for NO_3^-, 0,01 - 5 µM for NO_2^-, 0,1 - 1,10 µM for NH_4^+ and less than 10 µM for most of the organic forms,[87,86,93,102-195] with specific rate constants (K) (log_{10} unit/day) from 0,48 to 3,3 for NH_4^+, and 0,45 to 3,0 for NO_3^- and 0,4 to 1,7 for alternative nitrogen forms.[106]

The relative ratio of N to P is also important in maintaining optimal algal growth, although again the relative ratios may vary, Rhee and Yull[107] reported than N:P ratio of 30 was the boundary of nutrient limiting the growth of *Scenedesmus*, although Kunikane *et al.*[95] found neither N nor P to always be the only factor limiting the growth of *Scenedesmus*, rather a multiplicative effect, in the ranges 2-50 N:P. In general, at N:P ratios above 10, phosphorus becomes the limiting factor, and nitrogen at ratios below 10, with an optimal ratio of 10 - 25:1 for most algal species.[80, 106-112]

Phosphorus

The principal form in which phosphorus is assimilated by algae is as inorganic phosphate ($H_2PO_4^-$ + HPO_4^{2-}), although orthophosphate can be obtained from organic and inorganic polyphosphates of low molecular weight.[113,114] The uptake of phosphate is an energy dependent reaction, the energy being supplied by either photosynthesis or respiration, dependent upon the availability of light. Phosphate uptake is also closely related to the N:P ratios of the media and algal cells.[106]

Most algae fall into groups with a tolerance below 20 µg/ℓ P, with half saturation values for PO_4-P in the range of 0,01 to 16 µM.[87,93,107,115,116] Uptake kinetics are reported to either be proportional to ortho-P concentration in the medium when it is the limiting nutrient, or independent, supporting luxury uptake.[95,117] Certain species are able to activate enzymatic pathways and transport processes to enhance the extraction of low levels of phosphate from the water body as well as utilizing stores of phosphate from polyphosphate granules accumulated within the cells.[118] as high as 13% by weight, under nutrient rich conditions.

Carbon dioxide

Under conditions where adequate light, nitrogen and phosphorus are present, the growth rate of algae may be limited by the availability of suitable carbon sources.[40,75,119] The most acceptable form is as CO_2 although many algae can also utilize bicarbonate or organic compounds through mixotrophic pathways,[63,66,75,106,120] whilst the symbiotic association with heterotrophic bacteria is the more general source of CO_2. The minimum Km for CO_2 is reported to be 11 - 18 µM.

NUTRITIONAL VALUE

The nutritive value of algal protein is comparable, and in many cases superior to conventional protein feed supplements, in their gross protein content, amino acid quality and composition as well as nutritional acceptability and digestibility.[129-133] Table 1 summarizes some of the extensive data on algal protein profiles.

Table 1: Gross chemical composition of different algae (% of dry matter)[133]

Alga	Protein	Lipids	Carbohydrates	Nucleic acid
Spirulina platensis	46-50	4-9	8-14	2-5
	62,5	3,0	8,5	3,9
Spirulina maxima	65,0	2,0	20,0	2,9-4,5
	60-71	6-7	13-16	4-5
Chlorella vulgaris	51-58	14-22	12-17	
Chlorella pyrenoidosa	57,0	2,0	26,0	3-6
Scenedesmus obliquus	50-56	12-14	10-17	6,0
	52,0	9,0	12,5	
Scenedesmus quadricuada	47,0	1,9		
	57,0	6,0	32,0	
Dunaliella salina	49,0	8,0	4,0	
Dunaliella bioculata	63,0	11,0	15,0	5,0
Synechococcus sp.	39-61	14-20	14-18	
Euglena gracilis	28-45	22-38	25-33	1-2
Prymnesium parvum	41,0	3,8		
Hormidium sp.	45,0	1,1		
Ulothrix sp.	58,0	1,7		
Uronema gigas	51	1,2		
Stigeoclonium sp.				

The relative deficiency of the essential amino acids, tryptophan, methionine, lysine and cysteine is commonly identified in the algal proteins,[133]. It is also generally acknowledged that target organisms (fish, ruminants, monogastrics and man) differ quantitatively and qualitatively in their amino acid requirements as well as according to strain, sex and age.[129,133,135]

A more important criterion in evaluating the nutritional value may be obtained by determining the Total Digestibility Coefficient (TDC), the Nett Protein Utilization (NPU), Protein Efficiency Ratio (PER), and the Biological Value (BV).[133] In these cases short term feeding tests are usually performed on mice and rats. NPU = weight gain/N consumed x 100, PER = retained N/quantity of protein consumed. BV = absorbed N/N consumed - faecal losses, and DC = N consumed - faecal losses/N consumed. Table 2, illustrates a range of values that

have been reported. The variation is a response to differences in species, culture conditions, basal diets and more particularly, the processing of the algae prior to feeding.[131,133,136]

Table 2: Effect of processing on biological value (bv), digestibility coefficient (dc), and net protein utilization (NPU) of *Scenedesmus*, *Spirulina* and *Chlorella*.[133]

Algae	Protein (%)	BV	DC	NPU
Casein	10	87,8	95,1	83,4
Scenedesmus				
Drum-dried	10	80,8	81,4	65,8
Drum-dried	20	67,1	77,4	52,0
Sun-dried	10	72,1	72,5	52,0
Spirulina				
Sun-dried	10	77,6	83,9	65,0
Sun-dried + 0,3% met	10	79,5	91,9	73,0
Chlorella				
Protein extract	10	79,9	83,4	66,2
Protein extract	20	78,6	84,3	66,3
Protein extract + 0,37% met	10	91,1	86,1	78,4
Chlorella ^{15}N-method	10	83,5	79,0	65,9
Protein extract				
Egg	10	94,7	94,2	89,1

Table 3, illustrates the influence that processing has on the biological value, digestibility and nett protein utilization of certain algal species. Filamentous species tend to be less variable in these properties than unicellular algae due to differences in cell wall structure and the type of reproduction.[133,137-140]

Table 3: **Comparable data on biological value (BV), digestibility coefficient (DC), and nett protein utilization (NPU) of different algae[133]**

Alga	Method of processing*	BV	DC	NPU
Algae grown in synthetic media				
Scenedesmus	Air-dried	60,0	51,0	31,0
	DD	81,3	82,8	67,3
Chlorella	Air-dried	52,9	-	31,4
	DD	71,6	79,9	57,1
Coelastrum	DD	75,3	77,8	58,6
Uronema	DD	54,9	81,8	44,9
Spirulina	Air-dried			56,5
	Sun-dried	75,0	83,0	62,0
	Raw	63,0	76,0	48,0
	DD	68,0	75,5	52,7
Spirulina + 0,2% met	DD	82,4	75,7	62,4
Algae grown on sewage				
Scenedesmus/Chlorella (9:1)	Air-dried	54,3	65,4	35,5
	Autoclaved	54,5	65,5	35,6
	Cooked 30 min	56,0	73,0	40,9
	Cooked 120 min	48,7	69,8	44,0

* DD: Drum-dried

ALGAE AS AN ANIMAL FEED SUPPLEMENT

Poultry

The utilization of algae as a protein feed supplement has been extensively evaluated with poultry.[133] Mokady *et al.*[141] observed a significantly higher weight gain of broilers fed a 25% substitution level of *Chlorella* and *Euglena* or *Micractinium*, whilst a 50% substitution was only slightly inferior. In each case the feed efficiency was 0,5 attributed to a higher energy value of the algae than the control soybean meal. Shelef and Sandbank[142] reported drum-dried sewage grown microalgae could readily replace between 25 - 50% of the conventional soya protein in poultry feed, and can totally replace ground nut cake and fish meal in the feed for chicks, the predominant vegetable protein supplements in starter feeds.[143] Yannai and Mokady[144] reported no appreciable difference between a 15% level feed of *Micractinium* from a control fed to chicks, nor a 20% level fed to Japanese quails or mice, and Lipstein and Hurwitz[145] found no difference in egg production rate, food conversion and average egg weight of birds receiving up to 12% of their diet as algae, indicating that for laying birds algae can serve as almost the sole protein source.

Soeder[146] refers to experiments where levels of *Scenedesmus* above 25% substitution

Soeder[146] refers to experiments where levels of *Scenedesmus* above 25% substitution suppressed weight gain, except where 0,2% methionine was added, indicating that some differences in acceptability of algal species may be related to the individual deficiency in essential amino acids. Similar effects have been found where lysine[147] and vitamin B_{12}[148] were supplemented, lipids extracted,[149] and processing improved.[131,133]

An additional benefit associated with the supplementation of algal meal is the enrichment of the poultry meat and egg yolk from the carotenes and xanthophylls of the algae.[143,150-152]

Pigs

In the diet for pigs, microalgae has been found to be a suitable protein supplement up to a level containing 75% algal protein for rearing pigs, and as the sole protein supplement in fattening pigs, substituting for the conventional protein carriers such as barley and alfalfa,[153] although a level of 50% replacement would seem more appropriate. Lee (see [133]) reported sewage grown algae to replace 50% of soybean meal (15,5% of the diet). A similar value of 50% was reported by Taiganides,[154] and Yap *et al.*[155], for mature pigs and 33% algal replacement of the total meal for weaning pigs.

An acid hydrolysate of blue-green algae replacing up to 15% of the protein in diets for young pigs also resulted in comparable carcass yield and meat quality,[156] and Hintz and Heitman[148] included drum dried *Chlorella* and *Scenedesmus* at levels of 10% by weight, observing the algal meal was readily accepted giving a food conversion efficiency of 3,9 as compared to 3,85 for the control ration. *Spirulina* has been included in the starter diets of pigs at 12% until 42 days of age and thereafter a 5% diet in long term studies by Fevrier and Sevet.[157] No significant differences were observed in body weight and number and weight of the offspring born at the first and second lactations as compared to controls.

Viljoen *et al.*[158] investigated drum-dried, sewage grown algae at 10% inclusion in the diets of starter pigs. The food conversion rate was 1,99 as compared to 1,76 for sunflower oilcake and 1,77 for soybean meal. This was calculated to be the optimum inclusion rate in the pig growth meal, possibly as a result of the high ash content associated with the alum flocculation in harvesting. Inhibition of digestibility has also been reported by Koopman to limit the acceptability of algae to a 15% replacement level for finishing pigs.[5,159]

Ruminants

It has been suggested that the lower digestibility *per se* of algal biomass compared to conventional protein sources, is actually beneficial to ruminants in that the nitrogen is released further through the digestive system and is therefore more directly available for assimilation. Similarly, the relatively high levels of nucleic acids can be converted into allantoin in ruminants, which is unlikely to cause physiological problems in livestock, whilst the ability to breakdown the tough cell walls is a positive aspect of ruminant nutrition.[139,160]

Hintz and Heitman[153] reported a diet consisting of 60% algae 40% hay fed to lambs, sheep and cattle, to produce satisfactory growth and a protein digestibility of 72-74% in each case. Becker *et al.*[131] replaced groundnut cake at a 30% level by *Spirulina* in diets of calves and only observed an 8% reduction in body weight as compared to controls over a 6 month period, concluding that *Spirulina* could act as an ideal substitute protein for calf meal, as well as for fattening sheep.

Hasdai and Ben-Ghedalia[161] replaced 50% of the dietary nitrogen by alum-flocculated sewage grown algae in the diet of sheep as compared to a soybean meal control. The digestibility values of the dry matter were 69,3 and 79,3% respectively, and that of the organic matter 75,3 and 82,2%. The apparent nitrogen digestibility of the algal meal was 71,3% as compared to 83,0% for the control, although the amounts of retained nitrogen were the same. The lower digestibility was again considered to be due to the large amounts of aluminium carried in the biomass from the harvesting stage.

In order to reduce harvesting costs of the sewage grown algae, Sandbank[160] partially dried algal slurry by decantation and screening prior to mixing with milled straw at a rate to give a protein content of 15%. This was then sun dried and fed to sheep. Satisfactory feed intakes were achieved after 3 days of acclimatization, with an *in vitro* digestibility of 55,5% as compared to a urea based control of 60,1%. Another economical harvesting process is to feed wet fresh algal slurry directly to the animals, or use the algal pond effluent as drinking water for cattle, particularly important in feedlot enterprises where the recycling and optimal utilization of water is essential. Such an application has been investigated successfully for beef cattle in South Africa,[111,139] and beef and dairy cattle in Russia, where the microalgae were found to exert a positive effect on productivity.[162]

Human consumption

Spirulina produced principally in Mexico and Israel is already successfully commercialized in the health food market, where it is widely used to help people lose weight without being hungry or suffering malnutrition.[35,152]

Although there is a long history of the consumption of algae by humans, and the ready acceptance as a health food, there have only been a limited number of clinical tests to evaluate the nutritional or health associated benefits of algal biomass.[133] Soeder[132] refers to studies in Germany with drum-dried *Scenedesmus* fed to volunteers. A protein digestibility of 78% was recorded with a relative biological value of 81,5 - 96 of whole egg standard. The daily adult protein demand was estimated to be 60 - 80 g of algae. A level of 100 g/day resulted in sickness and indigestion, attributed to a lack of processing, low digestibility and unpleasant tasting nature of the algae concerned, as no toxicity was evident.[133] A Russian study with 50 - 100 g/day demonstrated no abnormalities over a period of three weeks, although a level of 150 g/day resulted in some allergic response and negative N-balance, though this did recover with accustomization.

Soeder[132] also refers to tests in Peru and Thailand, where only about 15 g/day of *Scenedesmus* was freely accepted, and then preferably in a dough or pastry, as with the traditional consumption of *Spirulina* in Africa at rates of 10 - 12 g/meal in sauces with tomatoes, beans, meat or fish,[163] and a level of 20 - 40 g/d *Spirulina* was acceptable to Mexican athletes with good results.[164] Gross *et al.*[163] reported good acceptability and weight gain, at rates of 10 g/day for adults and 5 g/day for school children, whilst undernourished children demonstrated a 27 g/day increase in body weight when receiving 10 g/day algae as compared to 15 g/day weight gain for a control group. Additional therapeutic factors in the algae were considered to be improving the health of the children rather than the algae protein supplement *per se*. Therapeutic and nutritional benefits of including algae in the diet have also been suggested as being particularly valuable to children between 2 to 5 years of age, pregnant women and nursing mothers.[133,166]

Aquaculture

Microalgae are extensively used as food for rearing larvae and juveniles of many species of commercially important molluscs, crustaceans and fish (freshwater and marine). They are also indispensable for culturing several types of zooplankton (rotifers, cladocerans, copepods or brine shrimp) which are used as live food in crustacean and finfish farming, and

providing essential minerals in the diet of freshwater fishes,[9,10,167,168] the most natural way of utilizing the biomass from algal ponds.

Meske and Pfeffer[169] performed detailed experiments on supplements of drum-dried algae fed to common carp, grass carp and mirror carp. The presence of algae up to 60% of the diet resulted in better growth as compared to conventional fish feed, with a food conversion efficiency of 2,31. Above the 60% incorporation mortality of the common carp and mirror carp increased, possibly as a result of high nucleic acid levels, whilst the grass carp increased in weight up to the 90% level where they had gained almost 3 times that of the control, at a food conversion efficiency of 1,34. Richmond and Preiss[170] refers to the successful incorporation of algae at a 12% level in carp feed and 40% for tilapia replacing up to 60% of the fishmeal or soybean meal component of the diet, in line with other reports of algae replacing 80 - 100% of the fish-meal component in fish diets, and of pure *Spirulina* increasing the growth of carp and tilapia 62,5% and 100% respectively. Saxena *et al.*[143] incorporated waste grown *Spirulina* at a 5% level to a polyculture growing in effluent from the cultivation pond to produce about 500 kg/260 m^2/y equivalent to 19,2 t/ha/y, comparable to extrapolated yields of 20 t/ha/y by Edwards[171] of tilapia harvesting algal pond effluents, and somewhat greater than the 8,1 t/ha/y reported by Turner *et al.*[172,173] for tilapia grown in seawater ponds.

Sandbank[174] reported that the replacement of 66% of the fishmeal by sewage grown algae produced an extrapolated yield of 8,47 t/ha/y, compared to the control of 7,14 t/ha, at a feed conversion ratio of 2,5. A similar conversion efficiency, 2,0, was reported for comparable studies in Israel.[175] Sandbank[174] also developed a process to produce semi-moist pellets prior to sun-drying. In replacing 70% of the fishmeal proteins 6,35 t/ha/y of fish polyculture were produced at a feed conversion of 2,75, as compared to 7,16 t/ha/y for the control at a conversion of 2,5.

TOXICOLOGY

A few species of freshwater microalgae, (mostly blue-green algae) have been identified as being potentially toxic.[176,177] However, almost without exception the animal feeding studies have indicated no direct toxicity of an algal meal. Lack of acceptability or digestibility is more a response to aesthetic factors such as taste and odour as well as deficiencies in essential amino acids, high levels of nucleic acids, and processing.[133,143,152,178]

Payer *et al.*[179] reviewed a series of investigations with the alga *Scenedesmus*, finding the algal meal toxicologically safe and nutritionally valuable, supporting other comparative

studies in India, Thailand, Peru, Mexico and Germany.[122,163,180] No toxic compounds could be detected in the tested algal strains for animals or humans, and all the relevant criteria were within recommended limits for utilization of single cell protein.

Ciferri[163] in reviewing the use of *Spirulina* in feeding tests found no reports of toxic effects or abnormality on post mortem observations of the consumer. These included long-term (18 month), multi-generation feeding trials and short-term massive feeding trials in which up to 800 mg/kg of body weight was administered orally for 12 days, and in mutagenicity tests with *Salmonella* and *Schizosaccharomyces*, Yannai and Mokady[144] investigated the toxicity of sewage grown *Micractinium* to chickens, quail and mice, and then the feeding of the chicken meat to rats in secondary toxicity testing. In none of four generations of the rats were abnormalities observed, and the chickens were considered fit for human consumption.

The meat, blood and tissue of fish cultivated either on algal pellets or in the effluent from algal ponds treating wastewaters have been found to be free of pathological contamination, whilst the external surfaces have been no worse than those from natural impoundments. The fish is considered safe to eat when due care has been taken in the preparation and cooking of the meat.[174]

There has been some concern expressed as to the high levels of nucleic acids present in microalgae,[133] although at the inclusion of algae in the diet at only 10 - 15 g/d, the nucleic acid levels are well within limits.[122,133,181]

Algae are also capable of accumulating pollutants such as heavy metals and pesticides, [182-184] which could be transferred to the consumer. Yannai and Mokady[144] reported low levels of absorbability of heavy metals from algae into the meat of quail, yet no abnormalities resulted, and Soeder[122] refers to reports of studies in Northern Thailand where the pollutant levels in the microalgae were lower than normally met within the vegetables sold at the German market.

PROTEIN MANIPULATION

The protein content of the microalgae may be manipulated or influenced by such factors as nitrogen supply,[80,100] light intensity and quality,[52] mineral concentration,[84] climate[122] and the age of the cells.[185] Since protein is predominantly nitrogenous in composition, the maximum protein contents and growth rates are proportional to the availability of nitrogen.[80,100,186] Protein content also tends to be inversely proportional to carbohydrate levels, and indirectly the nitrogen level.[185,187] Low nitrogen levels give rise to higher carbohydrate synthesis. A product containing more carbohydrate may be more valuable, in terms of

bioenergy conversion, where fermentation is more efficient at higher carbohydrate concentrations, yet lower nitrogen levels also tend to reduce growth yield.

CHEMICAL PRODUCTS FROM MICROALGAE

Microalgae synthesise a range of chemicals and pharmaceuticals that may be of commercial importance, including vitamins, carotenoids, amino acids, polysaccharides, antibiotics, steroids, lipids and growth substances.[11-15] Microalgae are an ideal vehicle for the commercial production of such chemicals because of their extraordinary biochemical plasticity which enables the control of their chemical composition by manipulation of the culture environment.[13]

The most advanced fine chemical production process using microalgae is that of ß-carotene from the halophilic green alga *Dunaliella salina*.[11,122] This alga can accumulate greater than 10% of its dry weight as ß-carotene which can realise about $1 000/kg in the health food market.[1,14] ß-Carotene and other carotenoid compounds used in the food industry are also produced by species of *Euglena, Haematococcus, Chlorella, Spirulina* and *Scenedesmus*.

Polysaccharides and hydrocarbons are another area of great potential. *Porphyridium* is cultivated for carageenan and agar, used in foods as emulsifiers, gelling agents and stabilizers, whilst *Isochrysis* and *Nanochloropsis* are being investigated as a source of hydrocarbon fuel oils. An algal produced bioflocculant and biodetergent has been developed as well as a biodetergent isolated from cyanobacteria, which may also find uses in the food industry.[11]

Other promising products from algae include the polyunsaturated fatty acids (PUFAs). These are essential dietary constituents for man and other animals, who convert them to a range of C_{20} compounds such as prostaglandins, prostacyclins and thromboxanes. One of the best producers of arachidonic acid is *Porphyridium cruentum* a red alga, which has been reported to produce about 0,2 g/ℓ/d.[11-15]

A wide range of pharmaceutically and biologically active molecules, is also produced by algae, including bacteriocides and fungicides, antihypertensive, anti-inflammatory and, antihelmintic agents, as well as growth promoting agents and related hormones.[11-15]

ECONOMICS

Information on the production costs of microalgae is limited to economic evaluations based on extrapolations from experimental systems, since no field scale facilities are yet in

operation for algal protein other than those dedicated specialist markets where the product commands a disproportionately high price. Consequently, prices quoted for microalgae range from more than $1 000/kg dry weight for monospecific cultures for use in hatcheries, and health food to $0,2 for harvested sewage grown algae.[9,35,37,186]

De Pauw and De Leenheer[9] refers to actual production costs of $4/kg in the summer to $23/kg in the winter for outdoor cultures of *Skeletonema*, similar to those quoted of $1 - 11,36/kg for *Spirulina*[2,152,188] $0,67 for *Chlorella*[3] and $1,75 for *Scenedesmus*.[189]

Soeder[232] evaluated the economics of algal cultivation for tropical and arid zone conditions. In the first instance an annual production of 45 t/ha was calculated to cost $1 562/t, reducing to $1 142/t for a subtropical production of 80 t/ha. Richmond[170] refers to two studies in Israel. In the first example a 10 ha plant on brackish water producing 750 t/y was calculated to cost $1 300/t. In the second for a 100 ha plant producing 80 t/ha/y, a fixed cost of $220/t was calculated and $450/t as the cost of production.

By combining the algal cultivation to wastewater treatment the overall costs of production are considerably reduced, and the process becomes more attractive as profit is recovered from the algal biomass of up to 71% of conventional treatment.[190-194]

Fox[166] calculated that an integrated system capable of meeting the needs of a rural village population of 500 should be possible for between $9 - 10 000. Similarly, Sandbank[174] estimated that for a population of 5 000 a system could be constructed for $100 000, where operating costs utilizing short term mixing (12 hrs/day), autoflotation and air drying would require an operating budget of only $35 000/y. The harvested algae mixed with milled straw as a lucerne substitute was estimated to generate $10 000/y profit, with water available for irrigational purposes, and aquaculture.

An advanced wastewater treatment study conducted in the Philippines[194] reported the total cost of production of the dried algal material including construction, running and harvesting costs at about $0,54/kg as compared to $2,2/kg for soybean or fishmeal. Shelef *et al.*[195] calculated at costs of $120/t of drum dried biomass, and less than $80/t for sun-dried, compared to the relative cost of soymeal between $180 - $230/t (on equal protein basis) or fishmeal at $280 to $400/equivalent tonne.

It is apparent that microalgal production is presently confined to the more expensive product market. A reduction of the price from the $1/kg to $0,4/kg has been considered to reflect a significant jump from the speciality market to that of bulk products.[192] Such a movement can only be accomplished with less sophisticated production technologies, the use of low value water resources (brackish or sea water), the integration of mass algal cultivation

in water treatment, aquaculture or irrigation. A positive advance seems to have been achieved in Singapore,[193] where High Rate Algal Ponds are reported to be required by law for the treatment of piggery wastes. Similar advances are urgently required worldwide to fully realise the tremendous potential of this high protein alternative.

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PART 2

MICROCYSTIS SCUMS FROM HARTBEESPOORT DAM AS A SOURCE OF FINE CHEMICALS

INTRODUCTION

Impoundments of South Africa are becoming increasingly eutrophic in character with resultant blooms of micro-algal species such as *Microcystis*. In some instances water from these impoundments is required for domestic and/or industrial use. This implies that some purification process precedes reticulation, and in cases of algal laden water an effective primary treatment generally involves flocculation/flotation or sedimentation. The resultant algal biomass is generally disposed of.

In comparatively recent times microalgae have been receiving increasing attention as a source of protein and unique medicinal and therapeutic fine chemicals of high commercial value. However, the economics of mass algal cultivation, harvesting and processing have generally militated against commercialization of the developed micro-algal technology.

The water treatment works at Schoemansville and Kosmos, both situated adjacent to Hartbeespoort Dam, a hypertrophic impoundment are ideally situated to capitalize on harvesting *Microcystis* as a byproduct of its water treatment function.

The aim of this research project was to analyze samples of algae collected from the dissolved air flotation unit at Schoemansville for fine chemicals over an annual cycle. Polynsaturated fatty acids, carotenes, phycocyanins, vitamins and proteins have a commercial value. If they could be extracted from the algal byproduct, the sale of the compounds could reduce the cost of water treatment.

METHODOLOGY

When the project was conceived, it was envisaged that algal samples from the DAF unit at Schoemansville and/or Kosmos would be collected on a fortnightly basis and analyzed for lipids, ß-carotene, α-tocopherol, vitamin B_{12} and amino acids. In addition to the above, it was intended to relate environmental factors to the concentrations of the compounds referred to above.

Unfortunately, during the period January to December 1989, *Microcystis* concentrations never reached levels recorded in the early to middle 80's. In fact there were periods when *Microcystis* was almost completely absent from the phytoplankton population (WATERTEK-unpublished data), a most unusual situation when compared with long-term phytoplankton observations since 1972. The reasons for the greatly reduced numbers of *Microcystis* and absence of scums are complex, but may be partly related to recent changes in the N:P ratios. Phosphate concentrations declined steadily from 476 µg/ℓ in 1984/85 to 134 µg/ℓ in 1987/88 a trend which has continued (Chutter, 1989). While the phosphate concentrations have

declined the loads of nitrogen species entering the dam have increased from 1395 ton/annum (1984/85) to 2109 ton/annum in 1987/88 as the stored water volume in the dam increased. Furthermore the phosphate-P concentrations in the surface waters (0,5 m) were low during the summer of 1988/89, sometimes as low as 5 µg/ℓ with the result that the N:P ratios rose to over 25:1 (Chutter, pers. comm.). These changes contributed to conditions not conducive to the development of high concentrations of *Microcystis*, and no scums developed.

Only traces of *Microcystis* were present in samples collected from Schoemansville and Kosmos plants during the research period; insufficient for chemical extraction and analysis.

Algal scum samples had, however, been collected by one of us (W Scott) during the period August 1984 up to and including May 1987. These samples were freeze-dried immediately after collection and sealed in plastic bags. Weekly or two-weekly samples collected in a particular month were pooled. All samples were stored in the dark at 4 °C. Twelve samples were available for analysis, seven collected during the Autumn months (March to May), three during the Winter (June to August) and one each during the Spring (September to November) and Summer. Environmental data from Hartbeespoort Dam were obtained from existing WATERTEK data bases (Table 1).

TABLE 1: Summary of environmental conditions prevailing during the months for which algae compositional data are available.

Months	Temp °C $\bar{x}$	Chlorophyll *a* mg/m³ $\bar{x}$	N/P-Ratio $\bar{x}$
August 1984	14,9	145,8	4,52
March 1985	23,9	79,5	1,73
April 1985	21,4	63,4	2,39
November 1985	21,6	31,7	11,4
March 1986	23,7	65,3	12,42
April 1986	21,0	40,9	4,45
May 1986	17,8	63,8	3,98
June 1986	13,9	43,3	14,09
July 1986	12,6	46,1	12,61
January/February 1987	24,4	79,6	14,99
April 1987	22,2	260,8	7,87
May 1987	19,2	19,9	14,72

RESULTS

Gross chemical composition

The gross chemical composition of the *Microcystis* scum samples collected during the period August 1984 up to and including May 1987 are presented in Table 2. The total lipid,

protein and vitamin B_{12} content varied little between samples, whilst the ß-carotene and α-tocopherol levels varied considerably. Thus in November 1985, ß-carotene was not detected in the collectred samples whilst in April 1986 a concentration of 0,979 mg/100 g was recorded. The variation in the α-tocopherol levels were even greater, ranging from undetectable (6 samples) to over 40,0 mg/100 g (2 samples).

TABLE 2: Gross chemical composition of the *Microcystis* scum samples collected during the period August 1984 to May 1987.

Sample Reference	Lipids g/100 g	Protein (N X6,25) g/100 g	Beta-Carotene mg/100 g	Alpha-Tocopherol (Vit. E) mg/100 g	Vit. B_{12} µg/g
August 1984	3,7	54,4	0,334	42,7	1,35
March 1985	3,6	50,7	0,389	8,4	1,24
April 1985	3,2	52,7	0,212	0,7	1,20
November 1985	3,7	57,7	nd	0,1	1,64
March 1986	3,7	53,5	0,350	3,2	1,25
April 1986	4,1	56,9	0,979	nd	1,22
May 1986	3,3	55,0	0,283	47,5	1,41
June 1986	3,8	54,3	0,650	nd	1,66
July 1986	3,8	55,4	0,250	nd	1,38
January/-February 1987	2,5	46,3	0,442	nd	1,17
April 1987	3,6	50,2	0,071	nd	1,32
May 1987	3,7	51,5	0,221	nd	1,25

Protein and amino acid composition

The crude protein content of *Microcystis* (Table 2) was obtained by multiplying the total nitrogen content by 6,25. This calculation overestimates the actual protein content and the nutritive quality of the protein as the latter is generally determined by the content, proportion and availability of its amino acids (Becker, 1988). The amino acid profile of the *Microcystis* scum samples is presented in Table 3. These values represent the mean and standard deviations. The amino acid profile compares favourably with other algae (Table 4) and as is common with most algae is deficient in the sulphur containing amino acids cystine and methionine (Leveille *et al.*, 1962).

Lipids

The total lipid fraction in the *Microcystis* samples ranged between 2,5 and 4,1 mg/100 g (Table 2). Although the fatty acid composition of *Microcystis* was not determined, Cyanobacteria tend to contain large amounts of polyunsaturated lipids with γ-linolenic acid being the major fatty acid (Becker, 1988).

TABLE 3: Variation in the amino acid composition of *Microcystis* samples collected from Hartbeespoort Dam during the period August 1984 to May 1987

Amino Acid	Recovery (% wet weight)		Range	
	$\bar{x}$	s.d.	max ^{m}	min ^{m}
Alanine	3,59	0,301	4,06	2,96
Arginine	5,18	0,482	6,40	4,66
Aspartine	5,31	0,368	5,87	4,55
Cystine	not	determined		
Glutamate	7,03	0,588	8,12	6,19
Glycine	2,07	0,580	2,52	0,28
Histidine	0,54	0,100	0,74	0,37
Isoleucine	2,66	0,232	3,17	2,25
Leucine	4,18	0,290	4,54	3,63
Lysine	3,10	0,380	4,04	2,56
Methionine	0,86	0,067	0,99	0,78
Phenylalanine	2,01	0,116	2,18	1,80
Proline	1,83	0,278	2,63	1,62
Serine	2,21	0,214	2,70	1,81
Threonine	1,18	0,166	1,47	0,95
Tryptophan	not	determined		
Tyrosine	1,91	0,135	2,16	1,67
Valine	2,70	0,330	3,05	2,20

FACTORS AFFECTING THE VARIABILITY OF THE COMPOSITION OF *MICROCYSTIS*

A number of factors have been shown to affect the vitamin content of algae. These include the genotype, the stage in the growth cycle, the nutritional status, the light intensity and other factors that affect the growth and metabolism (Carlucci *et al.* 1970; Odinstova *et al.* 1975; Nishijima *et al.* 1979; Shigeoka *et al.* 1979; Grün *et al.* 1984). The vitamin content of algae is known to vary greatly between species and also at times within species (Borowitzka, 1988). Koptera (1970) recorded vitamin B_{12} levels in *Microcystis pulverana* ranging from 0,41 to 6,65 µg/g while Kraut *et al.* (1968) reported α-tocopherol levels in *Scenedesmus obliquus* ranging from 140 - 1 000 µg/g.

TABLE 4: Amino acid pattern of selected algae including *Microcystis* scum (g(16gN)$^{-1}$)

	Ile	Leu	Val	Lys	Phe	Tyr	Met	Cys	Try	Thr	Ala	Arg	Asp	Glu	Gly	His	Pro	Ser	Ref
Recommended pattern by FAO	4,0	7,0	5,0	5,3	6,0		3,5		1,0	4,0									1
Egg	6,6	8,8	7,2	7,0	5,8	4,2	3,2	2,3	1,7	5,0	-	6,2	11,0	12,6	4,2	2,4	4,2	6,9	2
Spirulina maxima	6,8	10,9	7,5	5,3	5,7	5,0	2,3	0,7	1,5	5,6	9,0	7,2	12,2	17,4	6,6	2,0	4,1	4,9	3
Scenedesmus obliquus	3,6	7,3	6,0	5,6	4,8	3,2	1,5	0,6	0,3	5,1	9,0	7,1	8,4	10,7	7,1	2,1	3,9	3,8	4
Chlorella ellipsoidea	4,5	9,3	7,9	5,9	4,2	1,7	0,6	0,7	-	4,9	12,2	5,8	8,8	10,5	10,4	1,7	5,0	5,2	5
Dunaliella primolecta	5,5	11,1	5,6	5,3	5,4	3,7	1,9	0,8	-	5,5	7,5	6,1	11,3	11,7	5,8	0,5	-	4,7	6
Stigeoclonium	3,8	8,3	5,2	4,5	5,1	5,1	0,9	1,4	-	5,1	7,7	9,6	11,0	11,6	7,3	1,7	6,4	5,0	7
Microcystis	5,7	9,0	5,8	6,7	4,3	4,1	1,8	nd	nd	2,5	7,7	11,2	11,4	15,2	4,5	1,2	3,9	4,8	8

Reference 1. FAO/WHO (1973) 2. Diem & Lenter (1975) 3. Paoletti *et al.* (1980)
4. Becker (1984) 5. Priesly (1976) 6. Gibbs & Duffus (1976)
7. Batchelor unpubl. data 8. Batchelor *et al.* present study

TABLE 5: **Results of the stepwise regression analyses with ß-carotene and α-tocophophe as dependant variables and temperature, chlorophyll *a* and N/P-ratio as t independent variables.**

Dependant variable: ß-carotene				
Independent Variable	**Coeff.**	**Std Error**	**t Value**	**Sig**
Constant	0,5859	0,4634	1,264	0,2
Temperature	-0,0026	0,0210	-0,125	0,9
Chlorophyll *a*	-0,0001	0,0002	-0,980	0,3
N/P ratio	-0,0094	0,0176	-0,536	0,6
Dependant variable: α-tocopherol				
Independent Variable	**Coeff.**	**Std Error**	**t Value**	**Sig**
Constant	55,2622	25,2553	2,188	0,0
Temperature	-1,7741	1,1468	-1,546	0,1
Chlorophyll *a*	0,0034	0,0093	0,360	0,7
N/P ratio	-1,6935	0,9609	-1,762	0,1

In an attempt to explain the variation in the ß-carotene and α-tocopherol contents recorded in the scum samples (Table 2), a multiple regression analysis using selected environmental parameters (Table 1) was performed on the data. The resultant regression analyses (Table 5) showed no correlations between the variables tested. The lack of any significant correlation could possibly be attributed to:-

the disproportionate number of samples collected during the Autumn months (7) and Winter (3) compared to those collected during the Spring (1) and Summer (1),

the age of the scum prior to collection, particularly in the case of α-tocopherol, which has a known anti-oxidant function. Thus older scums with a long history of exposure to sunlight and depleted oxygen concentrations would be expected to have a reduced α-tocopherol content.

Additionally processing of algae after harvesting is known to affect the vitamin content (Table 6) although this fact cannot be used to explain the observed variability as all samples were treated in the same way.

TABLE 6: **The effects of processing on various constituents of microalgae. (Sources: Becker, 1984; Becker & Venkatamaran, 1984) (all concentrations in µg (g dry weight)$^{-1}$)**

	Scenedesmus acutus				*Scenedesmus obliquus*	*Spirulina platensis*
	Fresh	**Drum-dried**	**Freeze-dried**	**Cooked sun-dried**	**Drum-dried**	**Sun-dried**
B_1 (thimaine)	136	147	67	68	82	278
B_2 (riboflavin)	340	340	319	308	366	334
B_6 (pyridoxin)	-	-	-	-	25	13
B_{12}(cobalamine)	-	-	-	-	4,4	24
Biotin	-	-	-	-	2,0	6,0
Folic acid	-	-	-	-	7,0	-
Nicotinate	-	-	-	-	1200	-
d-Ca-pantothenate	-	-	-	-	166	-
Ascorbic acid	1653	177	598	205	177	-
ß-Carotene	501	211	171	15	2300	2300
Carotenoids	3490	1830	1650	570	-	-

MICROCYSTIS AS A SOURCE OF VITAMINS AND FINE CHEMICALS

Vitamins

The commercial potential of vitamins is great. In the US in 1981 the value of vitamins produced for human consumption was US 1,1 billion (US Department of Commerce, 1982). Estimates of the approximate values of vitamins and other fine chemicals obtainable from microalgae are listed in Table 7, Vitamins of particular commercial interest, which are produced by algae are vitamin B_{12} and vitamin E (α-tocopherol) (Borowitzka, 1988), the latter especially for use as an antioxidant (Kläui, 1976). A comparison of the vitamin B_{12} levels recorded in the scum samples with those present in other algae (Table 8) suggest that the vitamin B_{12} levels of *Microcystis* compare favourably with those recorded in *Spirulina maxima*, *S. platensis* and *Scenedesmus acutus*. The maximum levels of α-tocopherol in the *Microcystis* scums were, unlike the vitamin B_{12} levels, lower than recorded for other Cyanophyceae with the exception of *S. maxima* and *S. platensis* and other algae for which data are available.

Pigments

Microalgae form a number of accessory pigments such as phycobiliproteins and a

TABLE 7: Estimates of the value of various fine chemicals from microalgae (Values from the literature in Australian Dollars, US Dollars or Rand) (Source: A Jarvis, SASTECH)

Product	Comments	Price per kg					
		Aus$ Borowitzka 1985	Aus$ Borowitzka 1986	US$ Bennemann 1987	US$ Wilde 1988	US$ Borowitzka 1989	Rand CSIR 1989
Triglycerides Glycerol					1		2,5~4
Fatty Acid Arachidonic acid Eicosapentaenoic acid gamma-Linolenic acid			300 300 1800		150~1000 350	300	
Carotenoids beta-Carotene Xanthophylls Astaxanthin Canthaxanthin	Synthetic (pro vitA) from Dunaliella food supplement ‡1 food colouring ‡2 chicken feed	373~1770 2600 1150~2315	370~1780 1000~2000 2600 1150~2315	>500 300 200~500	500 100~300		1500
Pigments Phycobiliproteins Phycocyanin Phycoerythrin	research food colouring ‡3 "tagging agent" Porphyridium ‡4			>10 000 >100	>10 10 000/g	50	
Amino Acids Proline Arginine Aspartic acid				5~50 50~100 2~5	>20 5		
Polysaccharides	viscosifiers & gums			5~10		8	
Vitamins Vitamin A Vitamin B Thiamin (B1) Riboflavin (B2) Nicotinic acid Pyridoxine (B6) Pantothenate Biotin Folic acid Cobalamin (B12) Vitamin C Ascorbic acid Vitamin E Tocopherol		41 42 57 9 44 15 8850 150 5900 14 42	41 8800 5900 >10 42				

‡1 - It has recently been suggested that beta-Carotene has pharmacological value as an anti cancer agent and combatting the symptoms of AIDS. If so future demand is expected to rise.
‡2 - The value of beta-Carotene as an anti-oxidant is expected to rise ten-fold when tartrazine is banned.
‡ - PC from Spirulina has been commercialised as a blue pigment by DaiNippon Inc Co. of Japan, under the name "Linablue".
‡ - PE, the principal pigment of Porphyridium, would have a much larger potential market than phycocyanin due to the greater need for safe red pigments.

wide range of carotenoids in addition to the chlorophyll (chlorophylls *a, b, c* and c_2) (Borowtizka 1988). In some species the secondary pigments are present in greater quantities than the primary pigments. Of the pigments the carotenoids have particular commercial value as they are used as 'natural' food colourings as feed additive to enhance the colour of flesh of salmonid fish to enhance the colour of egg yolks and to improve the health and fertility of lot-fed cattle (Cohen, 1986). All of these markets have potential for expansion by supplying the carotenoids more cheaply or by supplying 'new' carotenoids (Borowitzka, 1988).

Of the over 400 known carotenoids only a small number are used commercially mainly as colouring matter; these include ß-carotene, lycopene, cryptoxanthin, zeaxanthin, astaxanthin and lutein, of which the first and last mentioned are the major commercial ones (Borowitzka, 1988). The limited number of commercially available (on large scale) carotenoids is considered to reflect the difficulties of the cost of synthesis rather than their lack of potential application.

At present only a single genus *Dunaliella* is known to accumulate up to 14% of dry weight as ß-carotene under nutrient stressed, high salt and high light conditions (Borowitzka *et al.*, 1984; Ben-Amotz *et al.*, 1980) whilst on average the carotenoids constitute 0,1% of the dry weight of microalgae (Borowitzka, 1988). The highest levels recorded in *Microcystis* during this investigation occurred during April 1986, with a value of 0,97 mg/100 g, a value considerably lower than that recorded for *Dunaliella*.

TABLE 8: Vitamin B_{12} and α-tocopherol (Vit. E) contents of various algae species (µg (g dry weight)$^{-1}$) (Source Borowitzka, 1988)

Alga	Vit. B_{12}	Vit. E
Cyanophyceae		
Anabaena cylindrica	0,63 - 1,1	4 000
Spirulina maxima	2,0	190
S. platensis	1,2 - 2,5	50 - 70
Microcystis pulverana)		
Nostoc punctiforma)	0,41 - 6,65	-
Anabaena hassali)		
Microcystic aeruginosa	1,17 - 1,66	1,0 - 475
Chlorophyceae		2 000
Chlorella vulgaris	0,06 - 0,07	526
Scenedesmus acutus	1,4	140 - 1000
S. obliquus	0,4	

Microcystis as a source of lipids

Microalgae may contain significant quantities of fats and oils with compositions similar to those of vegetable oils. The range of potential application of algae fats and oils is very wide and they resemble fish and vegetable oils. As such they could be considered as potential substitutes for petroleum products (Cohen, 1982) and substitutes for vegetable oil (Cohen, 1974).

Additionally as the lipids of some algae species are rich in essential fatty acids, they could be included in the diet of humans, animals and additives in feeds for aquaculture (Borowitzka 1988).

The range of lipid levels reported in various microalgae are presented in Table 9. The mean total lipid level recorded in *Microcystis* samples during the present survey was 3,6 mg/100 g, which falls in the range reported for the Cyanophyceae in Table 9. A number of factors are known to affect the lipid content of algae. These factors include the growth rate and various environmental factors, summarized in Table 10. It is evident from Table 10 that considerably higher lipid levels were recorded by Piorreck *et al.* (1984) for *Microcystis aeruginosa* during senescence than was recorded in the scum samples in the present study with the highest levels in the 'young' algae. The low levels recorded in the present study may thus reflect the age of the collected scum samples. At present only *Euglena gracilis* is cultured as a source of short chain saturated waxes of C_{28}, C_{27}, C_{29} and C_{30} chain length, with wax levels of up to 149,9% of cell weight (Inin *et al.*, 1983).

TABLE 9: Summary of the range of lipid levels reported in various micro-algae and the distribution of these lipids (on the basis of literature cited in text)

Algal class	Total lipids (% dry weight)	% of total lipid			Hydrocarbon (% dry weight)
		Neutral lipid	Glyco-lipid	Phospho-lipid	
Cyanophyceae	2-23	11-68	12-41	16-50	0,005 - 0,6
Chrysophyceae	12-72				
Prymnesiophyceae	5-48				
Cryptophyceae	3-17				0,0035
Xanthophyceae	6-16				2,8
Rhodophyceae		44	17	39	
Dinophyceae	5-36	41-58	42-59		0,004 - 0,2
Bacillariophyceae	1-39				0,2 - 0,7
Chlorophyceae	1-70	14-60	13-44	10-47	0,03 - 1,0
		21-66	6-62	17-53	(39,0)[a]
Euglenophyceae	17				

a High value for *Botryococcus braunii*.

TABLE 10. Effect of some environmental factors on lipid (% of dry weight) of a range of micro-algae

Environmental variable	Organism	Variable	% lipid	Reference
Light intensity	*Spirulina platensis*	10 --> 40 klx	4,2-6,2	Albitskaya *et al.* (1974)
	Phaeodactylum tricornutum	2,1 --> 21 klx	20,8-22,8	Orcutt & Patterson (1975)
Temperature	*Ochromonas danica*	15°C --> 30°C	39,9-53,3	Aaronson (1973)
	Chlorella minutissima	20°C --> 25°C	14,5-23,2	Seto *et al.* (1984)
Salinity	*Botryococcus braunii*	0 --> 6%	36-51	
$C0_2$ supply	*Scenedesmus acutus*	Low --> high	10-12	Dubinsky *et al.* (1978)
Nutrition	*Porphyridium cruentum*	Autotrophic *vs* heterotrophic	3,8-11,4	Becker & Venkatamaran (1982)
				Antia, Desai & Romilly (1970)
Senescence	*Chlorella vulgaris*	Young --> old	22-28	Collyer & Fogg (1955)
	Scenedesmus obliquus	Young --> old	19-32	Piorreck *et al.* (1984)
	Botryococcus braunii	Young --> old	23-34	Belcher (1968)
	Euglena gracilis	Young --> old	24-65	Piorreck *et al.* (1984)
	Navicula pelliculosa	Young --> old	14,5-19	Piorreck *et al.* (1984)
	Phaeodactylum tricornutum	Young --> old	24-29	Piorreck *et al.* (1984)
	Thallassiosira pseudononana	Young --> old	7,8-21,6	Fisher & Schwarzenbach (1978)
	Anacystis nidulans	Young --> old	14,4-10,2	Piorreck *et al.* (1984)
	Microcystis aeruginosa	Young --> old	19,0-16,5	Piorreck *et al.* (1984)
	Desert blue-green no. 92	Young --> old	9-26	Dubinsky *et al.* (1978)
	Chrysophyte F1	Log --> stationary	26,6-58,5	Barclay *et al.* (1985)
	Amphora sp.	Log --> stationary	11,7-25,7	Barclay *et al.* (1985)

Microcystis as a protein source

Microcystis aeruginosa and other bloom forming cyanobacteria are known to produce toxins which affect plants, zooplankton, birds, mammals including man and possibly fish (Scott 1987). It would thus seem inadvisable to consider using 'wild' harvested algal scums as a source of protein for inclusion in the diets of birds, fish or mammals, until more information on this subject is available. Fish, particularly Tilapia (*Oreochromis mossambicus*), exhibit fast growth rates in a *Microcystis* rich environment (Colman *et al.* 1990) and it may well be possible to include this algae in a ration.

ECONOMIC CONSIDERATIONS

The values of these fine chemicals (Table 7) suggest that the highest prices are obtained from ß--carotene from *Dunaliella*, astaxanthin, canthaxanthin and cobalamin (vitamin B_{12}). Algae are known to produce a wide range of fine chemicals, the most important of which and the assocated algae are summarised in Table 11. While the value of fine chemicals may be known, information on the production costs of microalgae is limited. Economic evaluations have been based largely on extrapolations from experimental systems. Soeder (1976) evaluated the economics of algae culture for tropical and arid zones where production costs were estimated to range between US $14 and US $34/ton. Richmond *et al.* (1980) estimated an algal cost of $1 300/ton from a 10 ha, 750 ton per annum plant in Israel. What is more interesting is the fact that a number of commercial mass cultivation operations have developed specifically around algae yielding high levels of ß-carotene, vitamin E, phycocyanin and algae which are marketed under health foods (Table 12). This tends to suggest that at present the costs associated with production, harvesting and separating algae and the fine chemicals tends to favour the exploitation of high priced commodities.

As the capital construction, operating and harvesting costs of intensive algal production units are high, the prospect of harvesting dense *Microcystis* blooms from an impoundment through a water supply system such as exists at Kosmos and Schoemansville seems an attractive proposition. However, an economic assessment based on an algal recovery rate of 5 kg/250 m^3/hour (estimated from figures provided by G Offringa, Table 13) with calculations based on the highest recorded fine chemical levels and prices, gives a daily rate of return of R54,16 excluding any processing or extraction costs).

TABLE 11: Summarized information from the literature on fine chemicals from algae (source: A Jarvis *in lett*)

Fine Chemicals	Algae
Triglycerides	
General	*Chlorella, Cyclotella cryptica*
Glycerol	*Dunaliella Tertiolacta, D. salina*
Fatty Acids	
General	*Spirulina* sp *Porphyridium* sp
Arachiodonic Acid	*Porphyridium cruentum, Oochromonas malhamensis, Prymnesium paruum, Poteriochromonas stipitata*
Eicosapentoic and γ-Linolenic acid	*Spirulina platensis*
Hydrocarbons	*Botryococcus braunii*
Carotenoids	
General	*Dunaliella salina, Phormidium* sp. *Isochrysis galbana*
ß-carotene	*Dunaliella salina, Spirulina subsalsa, Chlorococcum vulgaris Chlorella pyrenoidosa*
Astaxanthin	*Haematococcus plurialis*
Canthaxanthin	*Dunaliella salina, Chlorella zofingiensis Nannachloropsis oculata, Akinstrodesmus* spp. *Dunaliella psuedosalina, Chlorella zofingensis, Chlorella fusca, Akinstrodesmus* spp. *Chlamydomonas nivalis, Haematococcus pluvialis, Nannochloropsis* sp.
Pigments	
Phycobilliproteins	*Spirulina platensis, S. geitleri, Anabaena*
Phycocyanin	*cylindrica, A. variabilis*
Phycoerythrins	*Porphyridium sp.*
	Phormidium sp.
Polysaccharides	*Porphyridium cruentum, P. aerugineum Rhodella maculata, Chlamydomonas spp. Anabaena flos-aqua*
Vitamins	
Vitamin B	*Dunaliella salina*
Nicotinic acid	*Chlamydomonas eugamentos*
Riboflavin (B_2)	*Chlorella vulgaris*
L-Ascorbic Acid (C)	*Chlorella pyrendoisa*
α-tocopherol	*Porphyriduum cruentum, Dunaliella salina*
biotin (K)	*Porphyridium cruentum, Anabaena hassali, Dunaliella salina*

TABLE 12. Major commercial biotechnology operations using microalgal mass cultures

Company	Alga	Product
Cyanotech (American Cyanamid) Washington, USA * production facility in Keyhole Point, Hawaii	*Dunaliella salina* *Spirulina*	beta-Carotene & vit. E health food
Microbio Resources Inc. California, USA	*Dunaliella*	beta-Carotene
Earthrise Farms California, USA * Proteus maintain a joint venture with Dainippon (Tokyo) called Earthrise Farms	*Spirulina*	beta-Carotene phycocyanin
Betatene Ltd. Australia	*Dunaliella salina*	beta-Carotene
Western Biotechnology Ltd. Western Australia * contract with: ** Nihon Siber Hegner Ltd. (Tokyo) ** Dianippon Inc. & Chemicals Inc. (Tokyo)	*Dunaliella salina* *Spirulina* *Spirulina*	beta-Carotene phycocyanin for food colouring
Koor Foods Ltd. Israel	*Dunaliella salina*	beta-Carotene
Harima Chemicals, Japan	*Euglena*	unsat. waxes
Soso Texcoco, Mexico	*Spirulina*	health food

These calculations were based on the maximum recorded content of vitamin B_{12}, α-tocopherol and the pigment, ß-carotene. If one considers the variability recorded in specifically the α-tocopherol and ß-carotene contents during the survey period the daily rate of return would have been considerably lower than the calculated value.

TABLE 13: Calculation of the daily production value of fine chemicals from *Microcystis* at the Schoemansville and Kosmos plants *

Fine Chemicals	Max ^{m} conc. .	Max^{m} price R/kg	R/ton/*Microcystis*
Vitamin B_{12}	1,66 µg/g	16 343	R27,12
α-Tocopherol	47,5 mg/100 g	67	R31,83
ß-Carotene	0,97 mg/100 g	3 200	R31,32
		(a) total	R90,27

Estimated algal yield = 0,005 t/h per 1 Mℓ/day
Capacity of Schoemansville & Kosmos plants ± 5 Mℓ/d
.. Total algal yield = 0,025 t/h
Hourly fine chemical production value = R90,27 x 0,025 t/h total price ton^{-1} x hourly algal yield
= R2,25/h
.. Daily production value = R54,16

An additional complicating factor is the variable concentrations of *Microcystis* in the influent water to the purification plants. Negligible quantities of *Microcystis* are currently present in Hartbeespoort Dam resulting in extremely low recoveries of algae in the floats from the DAF units.

It would thus appear that under the present conditions and with the current price structure of fine chemicals that it would be uneconomical to use the Kosmos and Schoemansville facilities for harvesting *Microcystis* for fine chemical extraction.

A different conclusion may have been presented had the *Microcystis* concentrations approached the levels recorded in the early- to mid-eighties, a situation which may arise in the future.

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