EXECUTIVE SUMMARY

The main aim of this project was to extend the technology of waste-water treatment by microalgae using organisms which would -

(a) be cheap and easy to harvest so that the total suspended solids in effluent would conform to required standards; and

(b) reclaim nitrogen from the waste stream in such a form that the biomass produced could be used as a supplement to stock feed.

Systems employing a filamentous alga and a grazing invertebrate were compared and it was concluded that:

1 While 95% of the phosphate is released during the anaerobic fermentation of the stock waste, phosphate was undetectable in the blue-green alga, *Spirulina*, culture effluent at the N:P ratio used in the experiments.

2 The long-term maintenance of a stable *Spirulina*/zooplankton polyculture is technically feasible with minimal agitation. It was found that sufficient agitation could be supplied with a power input of 10 kW ha^{-1}.

3 The optimum salinity for the *Spirulina* was 10g NaCl/*l* and the growth of the organism was unchanged in a medium where the NaCl was replaced with an equivalent mass of Na_2SO_4. Thus, the use of underground waters high in sulphates is a feasible proposition. Calcium ions, however, inhibit growth.

4 The pH optimum of *Brachionus plicatilis*, the planktonic rotifer used in the polyculture, was similar to that of the *Spirulina*.

5 In freshwater systems, the fairy shrimp *Streptocephalus macrourus* showed optimal growth and survival and cleared the microalgae from the algal pond effluent when algae was fed at approximately 0,5 (dry mass: dry mass) of the estimated mass of fairy shrimps in the culture daily.

6 Optimal production was obtained from *S. macrourus* cultures when the fairy shrimps were held at approximately 1 000 organisms/*l* and the medium replaced at approximately 10 m*l* per organism daily on a continual basis.

7 The value of the *Spirulina* as a protein supplement would cover up to 97% of the running cost of the unit under the current (1988) economic climate, but not the capital amortisation. The value of the fairy shrimps, also as a protein supplement, will cover up to 54% of the running costs, but also not the capital amortisation under the current (1988) economic climate.

1 INTRODUCTION

Protein for inclusion in stock feed comes from a variety of sources, but the major sources over the past decades have been soy-bean oilcake and fish-meal. Soy-bean oilcake is a renewable resource, and as such the supply is predictable within the limits imposed by such factors as the vagaries of the weather. However, the fish from which fish-meal is made is a harvested resource. Even where the fish occur close to continents, the stocks can usually be exploited by fishing-vessels working in international waters, and it has proved very difficult to impose adequate legislation on nations fishing in international waters. The result of this is that many of the world's major known fish stocks are no longer large enough to give yields as high as they did during previous decades.

On the other hand, the world is faced with an increasing population, and most people prefer animal protein to plant protein. This increasing demand for animal protein has led, amongst other things, to the development of intensive livestock production units. A requirement of such units is the provision of a totally balanced diet for the livestock. The bacterial digestive processes of ruminants such as cattle, sheep and goats enable these animals to utilise a variety of nitrogen sources, including urea, to supply the required protein. However, monogastric animals (primarily chickens and pigs) are not able to manufacture their own protein, and so must be fed a diet which contains balanced quantities of the amino acids. Some of the essential amino acids are not found in any significant quantity in plant proteins, and, therefore, a certain amount of animal protein must be included in their diet. Thus, the demand for a suitable source of animal protein (currently fish-meal) will increase with the increasing demand for meat.

The projected shortfall of protein necessitates the investigation of alternate sources of protein for inclusion into stock feeds. One option is the industrial scale production of SCP (single celled protein). However, it may take twenty years for the technology to develop to a point where it will make an impact on the demand. As an intermediate measure, there is a possibility of exploiting further fish stocks off the west coast of Southern Africa. The estimated useful life of this stock is approximately twenty years (Cloete, 1986, unpublished). However, this source can only be relied on in the medium term and as long as there are no major political changes in the region.

Cottrell (1978) states that traditional economies treat the natural world and its resources with scant respect. He quotes K.E. Boulding as saying that mankind has operated a *cowboy* economy - like a reckless exploitation of limitless, empty plains - rather than a *spaceship* economy that is becoming increasingly necessary on a finite, small and crowded planet. In a *cowboy* economy, success is measured in terms of the amount of material turned over within the system and the earth is regarded as a free reservoir of resources as well as a bottomless rubbish dump. However, the criterion for success in a *spaceship* economy is the maintenance of the existing capital stocks in good order. By implication, this means a maximum recycling of used resources and a minimum input of new resources. Carpenter (1978) rates the recovery of the organic nutrients from wastes as one of the three world priorities, the other two being the search for alternate energy sources and the development of a universally acceptable family planning programme.

No provision has yet been made for reclaiming the usable nutrients from wastes. Waste streams from human or animal sources are too dilute for the economical production of yeast or bacteria, but they do contain nutrients which can be recycled. Lincoln and Hill (1980) estimated that approximately 70% of the nitrogenous compounds fed to pigs was lost either through spillage or excretion, and that if approximately 30% of this could be recycled as algal protein, then some 20% of the total protein requirement of the swine rations could be supplied by waste grown algae. This would reduce the units' dependence on outside sources of protein, much of which is obtained at an energy loss. For instance, the Japanese blue water fishing fleet runs at an overall efficiency of energy (whole fish) landed to energy (fossil fuel) expended of only 4% (Bardach, 1982). This inefficient use of finite fossil fuel resources is justified by the return in terms of essential amino acids contained in the fish.

If the algae grown in a waste water system was harvested by filter-feeding invertebrates, and these in turn were harvested for inclusion in stockfeed, then not only would the dependance on the harvest of natural fish stocks be reduced, but the provision of the essential amino acids would be less dependant on the inefficient use of fossil fuel. In fact the harvesting of solar energy by the algae during photosynthesis would render this process much more energy efficient than fishing.

The culture of filamentous algae such as *Spirulina* began at the University of the Orange Free State in mid 1983 using a defined medium (Zarouk's medium) in facilities designed for the culture of unicellular microalgae such as *Chlorella* and *Scenedesmus*. It was found that *Spirulina* could be grown successfully when agitated by a paddle-wheel, but that due to filament breakage, it was destroyed when agitated too vigorously. The algae was produced in blue plastic 'Portapools' of various diameters at a culture depth of ca. 1 m. These cultures were not agitated, and were harvested once weekly by pumping the entire culture through a net of 112 μm mesh size into an empty pond. Producing *Spirulina* in this way was costly because the medium was priced at ca. R16/m^3 in 1983, and the cultures were prone to invasion by unicellular algae.

During the search for an organism that could be used as a means to recover nutrients from waste waters, it was shown that *Spirulina* grew well in brack water to which animal wastes had been added. It was also shown that it was to the *Spirulina*'s advantange to be grown in polyculture with filter feeding invertebrates. These filter feeding invertebrates consumed the competing microalgae, and allowed the *Spirulina* to grow as a clean culture. Filter feeding invertebrates such as the brine shrimp *Artemia* and the water-flea *Moina micrura* were able to live successfully in *Spirulina* cultures while the *Spirulina* density was low, but rotifers such as *Brachionus plicatilis* and *Hexarthra fennica* were the only organisms able to live successfully in dense *Spirulina* cultures (Mitchell and Richmond, 1987). Therefore the development of a system for the recovery of wastes from intensive livestock units could, once the technology has been adequately developed, enable the enterprise to produce a protein supplement at a lower price than the conventional supplements.

Mitchell and Richmond (1987) has shown that a system comprising *Spirulina*, a filamentous algae, and filter feeding invertebrates (rotifers) was able to recover from heavy infestations of unicellular microalgae. The generalized response to a heavy inoculation of unicellular microalgae is shown in Fig 1.

The rotifer numbers increased rapidly to a point where the algae was consumed faster than the reproduction rate of the algae. Rotifer breeding ceased at about the time the unicellular algae disappeared from the culture and their numbers fell to low values before building up again to the steady state (ca. 1 to 2 cells/ml for *Brachionus plicatilis*). The *Spirulina* output lagged a little after the unicellular algal numbers had been reduced before it began to build up to a steady state.

The nutrient requirements of the *Spirulina* grown in an agricultural waste have been determined (Mitchell and Richmond, 1988). Using full strength Zarouk medium as control, it was found that solid waste produced by the animals on intensive livestock units at 1 g/*l* could replace all the nutrients in the Zarouk medium except for bicarbonate and nitrate. Under continuous light in the laboratory, addition of up to 2 g/*l* $NaHCO_3$ stimulated growth, but further additions had little effect. In the same way, increasing the quantity of $NaNO_3$ from 0 to 0,25 g/*l* resulted in increased growth, but further increases had little effect. When these experiments were repeated in 0,5 m^3 cultures out of doors, the concentrations at which growth showed little further response were 0,5 g/*l* $NaHCO_3$ and 0,05 g/*l* $NaNO_3$ respectively.

Becker and Venkataraman (1984) showed that if urine was used in conjunction with solid agricultural wastes, there was enough nitrogenous compounds to support growth at a rate where further additions of nitrogen sources made little difference. Therefore, in a farming situation where all the waste produced by the animals would be available, the addition of further nitrogen sources would not be necessary.

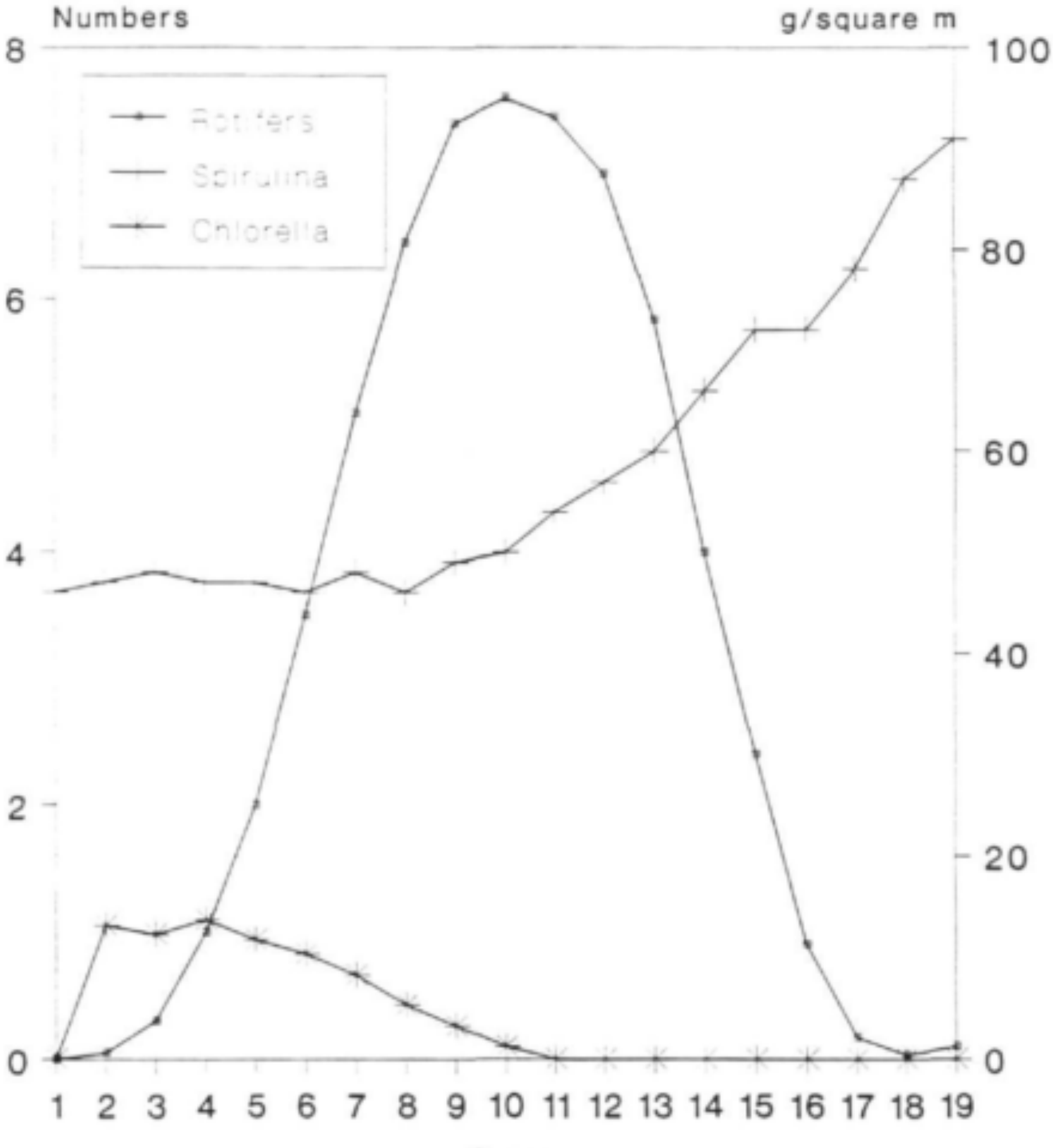

Fig 1 The response of Spirulina and rotifers when inoculated with Chlorella

This project was therefore aimed at extending the technology of waste water treatment by microalgae to produce an easily harvested end product which may be used as a protein supplement in stockfeed. While waste water may be effectively treated by microalgae to remove dissolved solids, the algae must be removed from the effluent before the effluent will conform to the required effluent standards for total suspended solids. Harvesting the algae by flocculation, centrifugation or any other such method renders the process too expensive. This project investigated the possibility of using organisms that were large enough to be harvested easily to treat the waste water. A dual approach was adopted:

- In the first place the filamentous alga *Spirulina* was used in a single step treatment system. As this alga requires brack water, its use is envisaged in areas of the country where there are sources of underground water which are too saline for conventional agriculture.

- The second possibility investigated was treating the waste water with microalgae and then using filter feeding micro-organisms to graze on the microalgae to remove the total suspended solids and at the same time convert it into animal protein which is easily harvested.

The micro-organism investigated during this project was the fairy shrimp (*Streptocephalus macrourus*), although others such as rotifers or cladocerans will perform the same function. The following questions were posed, some of which were answered from the literature and others from experimental work:

- How does the protein yield per volume of water used in aquaculture compare to the protein yield per volume of water used in conventional agriculture in South Africa?

- What contribution could the culture of *Spirulina* make to South Africa's projected protein shortfall?

- What production could be expected from algal mass culture?

- How effectively is waste converted to biomass?

- How does the conversion of algal protein to animal protein compare with the conversion of other forms of protein to animal protein?

- What is the economics of *Spirulina* production by the proposed system?

- How efficiently does anaerobic fermentation make nutrients available for use by the

Spirulina?

- How much agitation is necessary to maintain the stability of the system?
- What is *Spirulina*'s tolerance to Ca^{2+} and SO_4^{2-}?
- Is the pH tolerance of rotifers compatible to that of the *Spirulina*?
- If freshwater microalgae is cultured on the waste water and then fed to filter-feeding organisms, how efficiently is the microalgae converted to filter-feeder biomass, and how does the value of this compare with the value of the *Spirulina* produced from the same resource?