The effect of algal and bacterial filters on sea water quality during closed system culture

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The potential of using filamentous algae for biological filtration in closed culture systems was tested by comparing water quality changes in bacterial and algal filtration systems over a two month period. Juvenile *Penaeus indicus* Milne Edwards were cultured in 125-l recirculation systems and periodic analyses of inorganic nitrogen compounds (ammonia, nitrite and nitrate), total inorganic carbon, oxygen, major cation and trace metal concentrations were made. In both the algal and bacterial filtration systems, no significant changes occurred in the Ca^{++}, Mg^{++}, Na^{+} and K^{+}, and trace metal concentrations (Cu, Fe, Zn, Mn and Co). Depletion of inorganic carbon due to nitrification occurred in the bacterial filtration systems, whereas in the algal filtration systems inorganic carbon increased. An explanation for the latter occurrence is offered. The presence of algae did not give rise to oxygen shortages during periods of darkness. Although the filamentous algae used showed a reduced ability to take up nitrite, the build-up of ammonia and especially nitrate was effectively curbed. One of the only disadvantages of algal filtration appeared to be the lack of mechanical filtration of the water afforded by this method.

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Die potensiaal vir die gebruik van draadvormige alge vir biologiese filtrering in geslote sirkulerende sisteme is ondersoek deur 'n vergelyking te tref, oor 'n periode van twee maande, van die waterkwaliteit in alge en bakteriële filtrasie sisteme. Hersirkulerende sisteme van 125 l is vir die kweek van *Penaeus indicus* Milne Edwards gebruik en anorganiese stikstofverbindings (ammoniak, nitriet en nitraat), totale anorganiese koolstof, suurstof, hoof-katioon en spoorelementkonsentrasies is periodiek ontleed. Geen merkwaardige veranderings in Ca^{++}, Mg^{++}, Na^{+} en K^{+} is gevind nie. Desgelyks was daar geen verskil tussen die spoorelementkonsentrasies (Cu, Fe, Zn, Mn en Co) in die alge en bakteriële gefiltreerde sisteme nie. Uitputting van anorganiese koolstof weens nitrifikasie het in die bakteriële gefiltreerde sisteme plaasgevind, terwyl anorganiese koolstof in die algegefiltreerde sisteme vermeerder het. Daar word 'n verduideliking hiervoor gegee. Die teenwoordigheid van alge het geen tekort van suurstof gedurende periodes van donkerte veroorsaak nie. Alhoewel die draadvormige alge wat gebruik is 'n verminderde vermoë vir nitriet-absorbering getoon het, het dit die opbou van ammoniak en nitraat effektief tot stand gebring. Die gebrek aan meganiese filtrering van water getoon deur dié metode, blyk een van die min nadele van algefiltrering te wees.

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Little is known about the effects of algae on water in which animals are grown even though they have been a decorative feature of marine aquaria for decades (Atz, 1949, 1950). Alderson & Howell (1973) and Siddall (1974) have used unicellular algae for the removal of nitrogenous compounds from their culture systems. Goldman, Tenore, Ryther & Corwin (1974a, 1974b) used algae in a tertiary water treatment system, while Honn & Chavin (1975) employed algae in a quarternary sea water processing system. Recently, Naegel (1977) combined the production of fish and algae in recirculated fresh water. The present status of algae filtration is however, best summarized by Kinne (1976) who states that 'our present knowledge of the functioning and usefulness of algal filters is very limited.'

In the present study the effects of algal and bacterial filtration on water quality are compared. Filamentous algae used during the culture of *Penaeus indicus* in closed systems were of the genera *Rhizoclonium* Kuetzing and *Chaetomorpha* Kuetzing. The parameters of water quality monitored were inorganic nitrogen compounds (ammonia, nitrite and nitrate), total inorganic carbon, oxygen, major cation and trace metal concentrations.

Materials and Methods

Algal filtration systems and bacterial filtration systems (i.e. sub-gravel filters) of the type used by Spotte (1970) were set up in duplicate. Black polythene tanks (780 mm × 580 mm × 480 mm) of 125 l capacity were used.

In the bacterial filtration systems perforated false bottoms of fibreglass (GRP) sheeting supported a 10 cm layer of 3 – 5 mm quartsite gravel chips and oyster shell chips. In the algal filtration systems 120 g drained wet weight of filamentous algae were housed in 710 mm × 540 mm GRP trays supported above the water level in the tank. Growth of the filamentous algae was not monitored, but harvesting was periodically carried out when it appeared that the algae were becoming very dense (±450 g drained wet weight). Water was circulated by airlift at a rate of approximately 2 l min^{-1}. The two types of systems are illustrated in Figures 1a and 1b respectively.

Two experimental trials were run, the second primarily to obtain data on nitrate concentrations, the analysis of which was unsatisfactory in the first experimental trial. The filtration systems were stocked with twenty juvenile

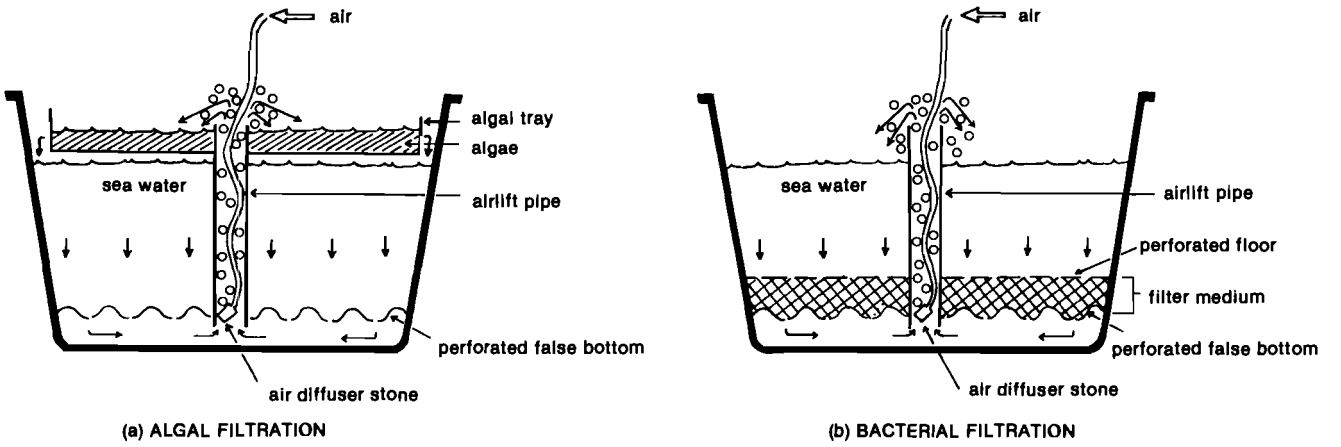

Figure 1 Diagram of (a) an algal filtration system and (b) a bacterial filtration system used in the experimental trials. Arrows indicate the direction of water flow.

Penaeus indicus Milne Edwards of 0,5 g wet weight (i.e. 28 g wet weight m^{-2}). This biomass loading increased to approximately 200 g wet weight m^{-2} during the two-month duration of the experiments. Both experimental trials were carried out in a constant environment room set at 28 °C (±1 °C) and with a 12:12 hour light-dark regime. The incident light received by the algae was 657 – 805 watts m^{-2} while that received by the prawns in both systems was adjusted to 1000 – 2000 lux. The prawns were fed twice a day with a pelleted ration at a rate of 2,5% of their wet weight.

Ammonia and nitrite analyses were carried out three times a week according to the methods of Solarzano (1969) and Strickland & Parsons (1968). Nitrate was determined by the reduction method of Mullin & Riley (1965). Total alkalinity (expressed as meq $CaCO_3 l^{-1}$) was determined every 7 – 10 days according to the method of Mackereth (1963). Total alkalinity, pH, temperature and salinity were then substituted into the equations of Mook & Koene (1975) and Edmond & Gieskes (1970) to calculate total inorganic carbon. The light-dark fluctuations in the percentage oxygen saturation in the algal tray inlets and outlets were measured every three hours over a 52-h period using a Radiometer BMS 3 Blood Gas Analyzer. The concentrations of the major cations (Na^+, Ca^{++}, K^+ and Mg^{++}) were measured fortnightly using a Varian Techtron Type AA-4 Atomic Absorption Spectrophotometer. The same apparatus was used to determine trace element concentrations (Cu, Fe, Zn, Mn and Co) after extraction from the sea water using the sodium diethyldithiocarbamate-chloroform technique outlined by Watling & Watling (1976). With the exception of the inorganic nitrogen compounds, results from replicate systems have been averaged before plotting them graphically. Water losses due to evaporation were daily compensated for by the addition of de-ionized water.

Results

In Figure 2 the results of the major cation values have been plotted. No apparent changes occurred in the Na^+, Ca^{++}, K^+ and Mg^{++} concentrations in either the bacterial or algal filtration systems. The small changes that did occur, fell within approximately 5% of the initial concentrations in normal sea water. Similarly, except for

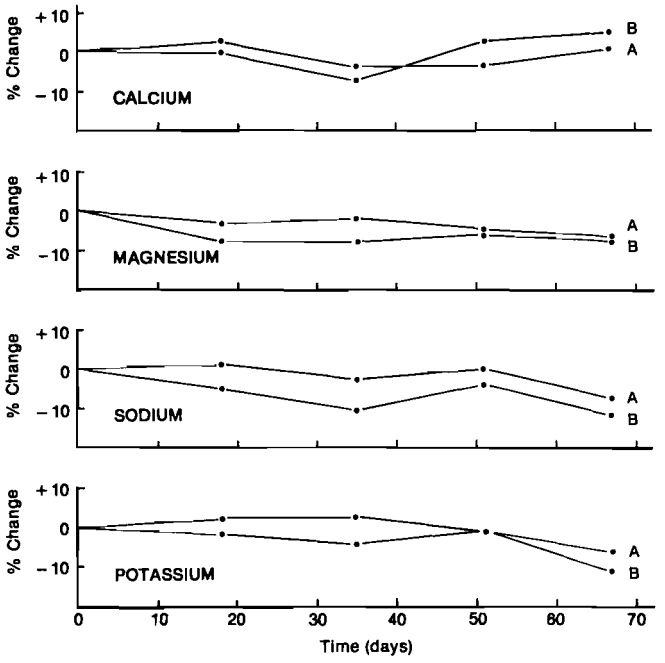

Figure 2 Percentage change from natural sea water of major cations in the algal (A) and bacterial (B) filtration systems.

Cu concentrations, little change occurred in trace metal concentrations (Figure 3). In both systems, there appears to be an overall increase in Zn concentrations. Also, over the first 34 days there was a small depletion of Fe. Mn and Co have not been plotted since changes in concentration were so small. The concentration of Cu in the bacterial filtration systems increased from 3,2 μg Cu l^{-1} to 32,3 μg Cu l^{-1}. This build-up was curbed in those systems containing filamentous algae, since Cu levels in the algal filtration systems only increased from 1,6 μg Cu l^{-1} to 10,0 μg Cu l^{-1}. The increase in Cu concentration was thought to be due to condensation moisture in the copper piping used in the air reticulation system of the laboratory building. To remove this moisture, a 'Compair' Model M4 automatic water-ejector and filter was installed into the airline system for the second experimental trial. Cu levels were determined at the beginning and end of this experiment and no increase was found in the culture water of the biological filtration systems tested.

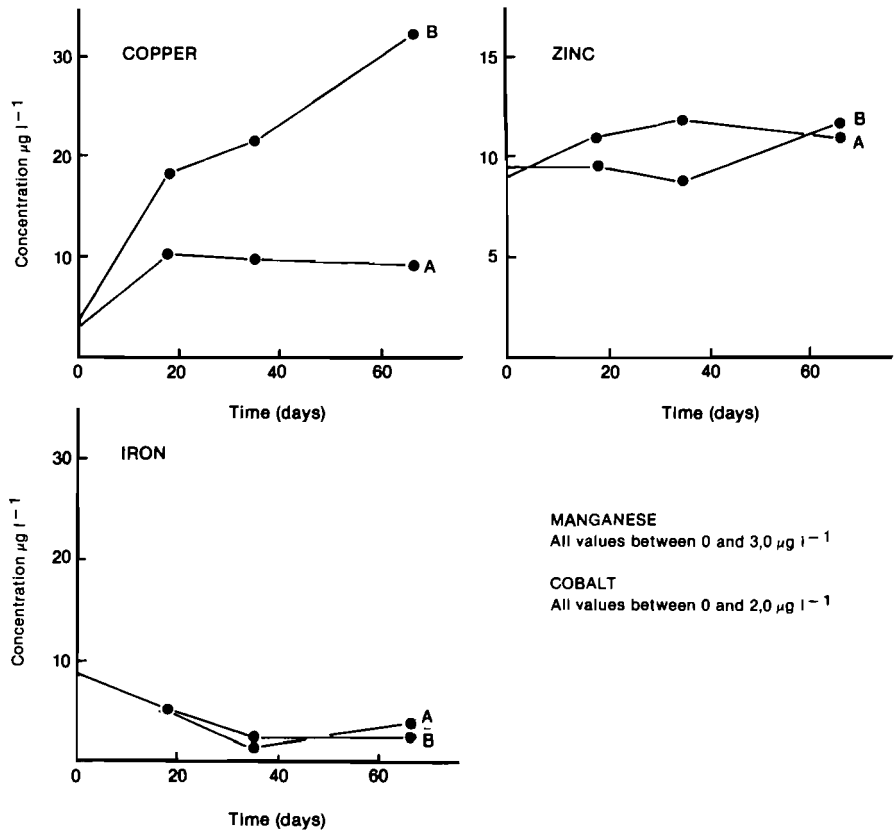

Figure 3 Changes in trace metal concentrations in the algal (A) and bacterial (B) filtration systems.

In both experimental trials the total inorganic carbon content of the water dropped in the bacterial filtration systems but increased markedly in the algal filtration systems (Figure 4). Light-dark fluctuations in oxygen content in the algal filtration systems are shown in Figure 5. The oxygen concentrations at the outlet of the trays were marginally higher than those at the inlet during periods of illumination. The reverse occurred during periods of darkness. The concentrations of ammonia, nitrate and nitrite found in the second experimental trial are shown in Figure 6. Nitrification occurred in both systems, although only the bacterial filtration systems were provided with gravel. The maximum concentrations of ammonia and nitrite occurring during the establishment of nitrification in the bacterial filtration systems (Figures 6a and b) are lower than in the algal filtration

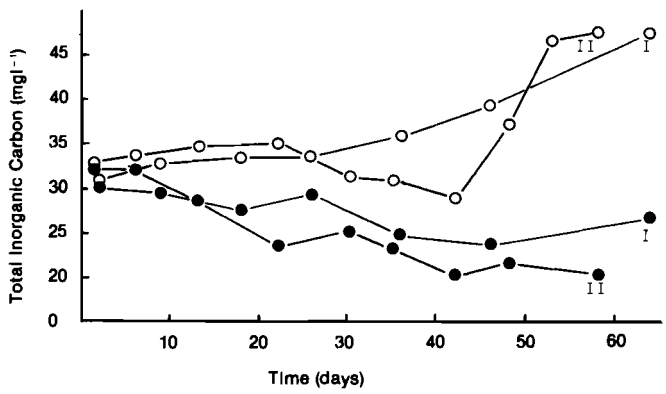

Figure 4 Total inorganic carbon levels in the algal filtration systems (——O——) and bacterial filtration systems (——●——) during the first (I) and second (II) experimental trials.

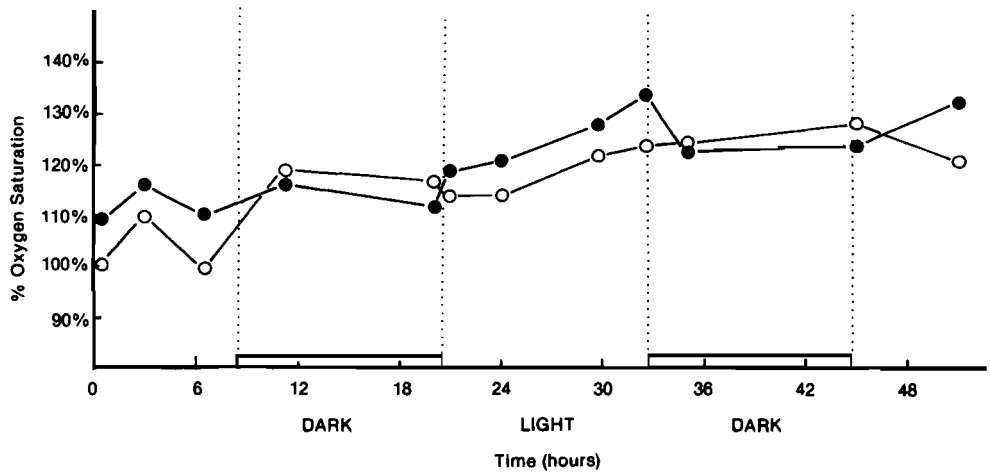

Figure 5 Light-dark fluctuations in percentage oxygen saturation in algal tray inlets (——O——) and outlets (——●——) measured over a 52-h period.

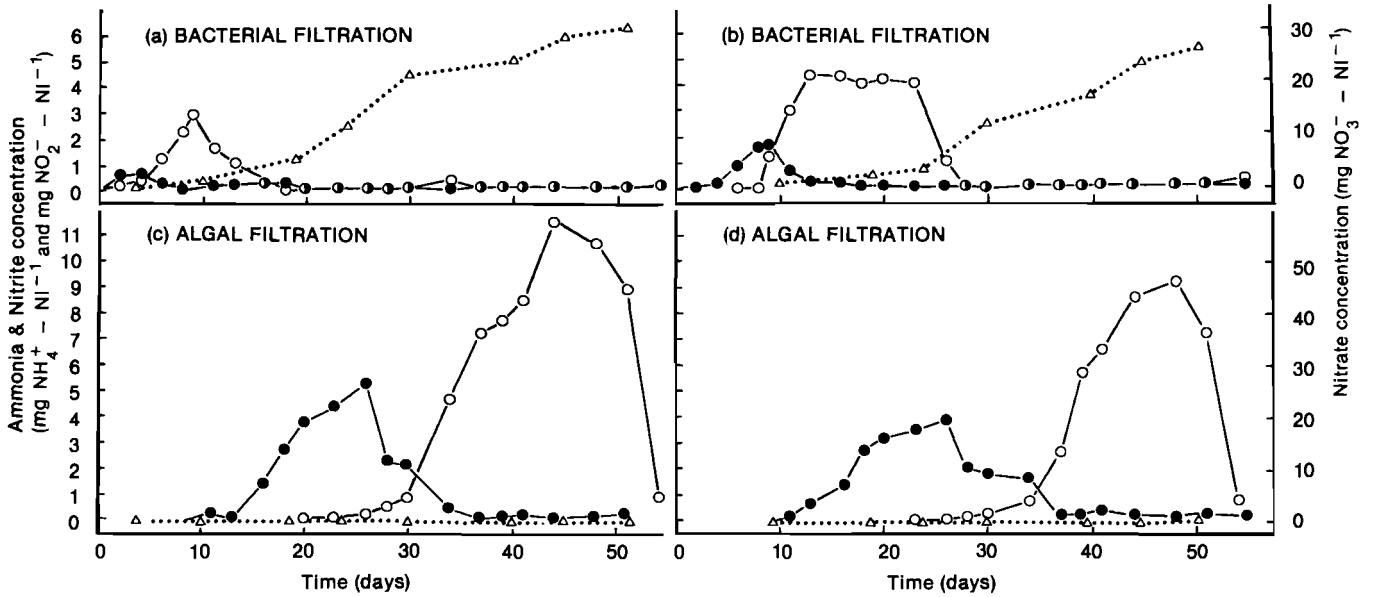

Figure 6 Ammonia (—●—), nitrite (—○—) and nitrate (....△....) concentrations in the bacterial filtration and algal filtration systems.

systems (Figures 6c and d). Furthermore, the establishment of nitrification was completed in 18 – 28 days in the two bacterial filtration systems, whereas nitrification took the full duration of the experiment (55 days) in the algal filtration systems. Nitrate concentrations increased steadily in the two bacterial filtration systems reaching 26,2 and 30,9 mg NO_3^- – N l^{-1} after about 50 days. No increase in nitrate concentrations occurred in the algal filtration systems.

Discussion

It would appear that major cation changes do not occur during short-term (two month) culturing in closed systems. The accidental copper contamination in the first experimental trial illustrated an important advantage of algal filtration. The resultant copper levels in the bacterial filtration systems showed a tenfold increase, whereas in the algal filtration systems, this increase was prevented. Little change occurred in the concentrations of the other trace metals. Although Siddall (1974) furnished no data, he stated that in his culture system a 'mixed algal population (predominantly *Chlorococcum* species) removed all trace elements analysed in significant amounts'. Periodic harvesting of these algae then gave rise to a net loss of trace elements from the water in the system. In these algal systems no such depletion occurred and it is felt that a sufficient replenishment of trace metals occurred with the introduction of food.

The changes in total inorganic carbon levels in the algal and bacterial filtration systems are of interest. The drop in total inorganic carbon in the bacterial filtration systems can be explained by the bacterial requirement of inorganic carbon during nitrification, according to the formulae of Haug & McCarty (1972):

$$55NH_4^+ + 5CO_2 + 76O_2 \xrightarrow{Nitrosomonas} C_5H_7O_2N + 54NO_2^- + 52H_2O + 109H^+$$

$$400\,NO_2^- + 5CO_2 + NH_4^+ + 195O_2 + 2H_2O \xrightarrow{Nitrobacter} C_5H_7O_2N + 400NO_3^- + H^+.$$

Furthermore, the production of H^+ ions during nitrification lowers the pH and upsets the carbonate-carbon dioxide equilibrium in the water, resulting in a further loss of inorganic carbon to the air (Wickins, 1976). This loss of inorganic carbon can be deleterious to Crustacea. Greenaway (1974) has shown that the absence of external HCO_3^- reduces the net uptake of calcium in postmoult freshwater crayfish, and Wickins (1976) found that low inorganic carbon levels (<10 – 12mg l^{-1}) were likely to be lethal to prawns.

In both trials there were marked increases in total inorganic carbon concentrations in the algal filtration systems (Figure 4). The explanation offered is that CO_2 produced from respiration of the algae, prawns and micro-organisms (decomposition of excess food and faeces) exceeded CO_2 taken up during photosynthesis. However, this excess of free CO_2 itself would not increase the inorganic carbon levels, since from the methods it can be seen that the increase was monitored as an increase in titratable base and not free CO_2. Carbonate and bicarbonate ions can be derived from three sources: (1) the reaction of free CO_2 with water, (2) the reaction of mineral carbonates with free CO_2 and water, and (3) bacterial reduction processes (Spotte, 1970). The first alternative can be excluded since at the pH range that occurred during these experiments (7,65 – 8,18) this reaction is not dominant. The second alternative can also be excluded since it is felt that although mineral carbonates could be considered as abundant in the bacterial filtration systems, this was not the case in the algal filtration systems. It is thus suggested that the free CO_2 reacted with water and unionised ammonia to produce carbonate and bicarbonate ions as described by Berner (1968):

$$CO_2 + NH_3 + H_2O \rightarrow NH_4^+ + HCO_3^-$$
$$CO_2 + 2NH_3 + H_2O \rightarrow 2NH_4^+ + CO_3^=$$

Should this explanation be correct, it would confer a major advantage upon the use of algae for biological filtration purposes. Toxic unionised ammonia would be rendered less harmful, and poor growth and survival due to depressed levels of inorganic carbon would be avoided.

The photosynthetically-induced light-dark reversals in the % oxygen saturation at the outlets and inlets of the algal trays (Figure 5) were to be expected. Of importance, however, is that the drop in oxygen concentrations at night were minimal and in no way limiting to the animals cultured. Oxygen levels remained in excess of 100% saturation. Similar results were obtained by Alderson & Howell (1973) who recorded oxygen values of 125% saturation during the light period in rearing tanks containing unicellular algal populations. No explanation can be found for the progressive increase in oxygen saturation which occurred over the 52-h period.

Nitrification occurred in the algal filtration systems (Figures 6c and d). This was obviously due to the colonization of nitrifying bacteria on the walls of the tanks. However, the capacity of the systems with gravel (Figures 6a and b) to undergo nitrification in a shorter time period and to maintain lower ammonia stabilization levels than those with algae (Figures 6c and d) was probably due to the greater surface area offered for bacterial attachment by the gravel chips. The earlier stabilization of nitrite levels in the bacterial filtration systems suggests that algae might not possess a large capacity for nitrite uptake.

Nitrate did not accumulate in the algal filtration systems, whereas in the two bacterial filtration systems the final concentrations were 26,2 mg $NO_3^- - N\ l^{-1}$ and 30,9 mg $NO_3^- - N\ l^{-1}$. The filamentous algae thus effectively removed all nitrate produced in the algal filtration system. Further work is required to ascertain whether algae could curtail the exceptionally high nitrate concentrations (1208 ppm) found by Otte & Rosenthal (1979) in their high density fish culture experiments.

A significant disadvantage in the use of algal filtration systems was the lack of mechanical filtration which allowed excess food and faecal material to be carried up the airlift pipes and deposited in the algal trays. The subsequent decomposition of this material was probably the cause of the eventual decay of some sections of filamentous algae. A possible solution whereby the above disadvantage could be avoided, while maintaining the previously mentioned advantages of algae (uptake of nitrogenous compounds, supply of inorganic carbon, buffer against contamination etc), is to combine the two filtration methods. Alternatively, algae could periodically be added to a bacterial filtration system when it is noticed that inorganic carbon levels are low, or that nitrate levels are high.

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