Improvement of Algae Settleability in High Rate Ponds Using Rotifers at Richmond Field Station, U.C. Berkeley

Tien Dung Ho

Abstract Rotifers offer a natural and efficient means to improve algae settleability in high rate pond systems, a wastewater treatment system, through their selective grazing on smaller algae (1-10 micrometers). However, the survival of the rotifers in these water systems is highly dependent upon pH and ammonia concentrations. Overgrazing on algae results in increased ammonia levels and pH which are toxic and inhibit rotifer growth. An experiment was carried out to study improvement of algae settleability as well as rotifer sensitivities to pH and ammonia, using samples of high rate pond water with different added rotifer concentrations and a control with no rotifers. Ammonia, pH, turbidity, rotifer and algae populations were monitored at four time intervals (0, 2, 4, and 7 days). The results indicated a significant improvement in algae settleability with increased rotifer populations. However, due to the rapid reproduction of rotifers, overpopulation of rotifers resulted in overgrazing on small algae which altered the water characteristics, pH and ammonia. Overpopulated treatments experienced a die off of rotifers caused by excessively high ammonia concentrations which reduced algae settling.

Introduction

Worldwide water consumption is a growing concern because it has increased eightfold over the past century (Gleick, 1998). Freshwater resources are especially limited because most of it is frozen in ice caps. The remaining freshwater resources, mostly groundwater, are not fully accessible since much of it is too deep to economically exploit (Alvarez 2000). Therefore there is a strong need to protect and conserve our water resources.

Wastewater treatment offers a practical solution to resolve our water needs. In 1972, the Clean Water Act passed to provide funding to create effective wastewater treatment methods while setting pollution discharge and primary water treatment standards. In 1977, the act was amended to require secondary treatment of wastewater to include biochemical oxygen demand and total suspended solids to all municipal wastewater before discharge. Many community wastewater plants experienced difficulties in meeting these stringent effluent requirements due to cost, reliability, and maintenance (USEPA 1998). Conventional secondary treatment systems include oxidation ponds, aerated lagoons, trickling filters, and activated sludge systems, with each system having its own limitations such as land requirement, cost, and effluent characteristics.

An innovative treatment method, the high rate pond, is cost effective and capable of producing effluent that is low in dissolved organic material, metals, nutrients, and hazardous bacteria that meet secondary treatment requirements. High rate ponds are shallow (20-50 cm) ponds designed in a racetrack configuration and is continuously mixed to reduce land requirement and maximize algae production. High rate ponds rely on the process of photosynthetic oxygenation to treat wastewater (Oswald 1988).

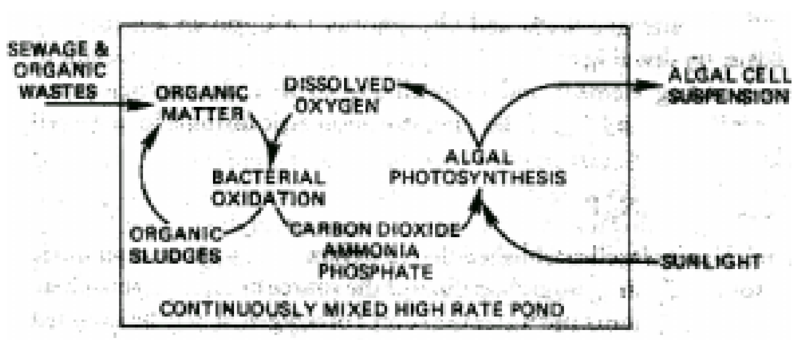

Figure 1: Cycle for Photosynthetic Oxygenation

In photosynthetic oxygenation, aerobic bacteria and algae share a symbiotic relationship in which bacteria depend on algae for oxygen, while algae depend on bacteria for nutrients. The bacteria oxidize the entering organic wastes to produce carbon dioxide, ammonia, phosphate, and bacterial biomass. The algae fix these organic wastes which are then volatilized or precipitated out of the wastewater (Puskas 1994). Also, the algae remove heavy metals by using its affinity for polyvalent cations to improve settling (Oswald 1988). By converting light energy to heat and by increasing pH, disinfection of coliform bacteria is increased dramatically (Cromar 1996).

After filtration to remove algae, high rate ponds have been shown to reduce BOD and suspended solids below required standards. In comparison to conventional treatment methods, high rate ponds require half as much land as oxidation ponds, have shorter residence times and lower evaporation losses, lower aeration costs than mechanical systems, and require little maintenance and no chemical disinfectants (Esen 1991). In addition, high rate ponds produce large amounts of algae that can be used as soil conditioners or fermented to produce methane for energy production (Rovisora 1996).

High rate ponds provide a promising wastewater treatment method by reducing bacteria, BOD, and nutrients, however the effluent is rich in small algae (less than 10 micrometers) due to slow separation in the liquid phase. As a result the effluent does not meet water quality criteria on suspended solids. Conventional solids removal techniques are applicable to high rate ponds but are expensive (50-60% of the total cost) and difficult (Esen 1994). For instance, polymer coagulants can be used to neutralize the charge on particles to improve settleability of the smaller algae. Microscreens and sand filters have been developed to remove most of the algae however, throughput rates are too slow and there are problems with clogging due to smaller algae (Esen 1991). Bioflocculation, autoflocculation, and sedimentation are capable of producing effluent that meet secondary standard but are often inconsistent. Due to the ionic charges and small size of algae such as *Chlorella* and *Chlorococcum* (2-3 micrometers), they remain suspended in the effluent for long periods of time.

Zooplankton grazing offers an alternative solution to improving algae settleability. Studies on rotifers (*brachionus calyciflorus*) reveal that they selectively graze on smaller algae ranging from 1 to 10 micrometers (Hansson 1998). After digestion, rotifers produce fecal pellets, which are composed of many smaller algae lumped together, called flocs. This drastically reduces suspended solids in wastewater since the fecal pellets are larger and settle significantly faster.

Because rotifers are capable of reproducing at rapid rates they often result overgrazing of algae. An initial population of 100 rotifers can increase to 10, 000 in ten days (Hoff 1997).

Overgrazing on algae significantly alters water characteristics, specifically pH and ammonia. Previous research shows that rotifer survival and reproduction are extremely sensitive to pH, temperature, and ammonia concentrations (Schluter 1985), however little else is known about these organisms.

The purpose of this research project is to assess the improvement of algae settleability in high rate ponds populated by rotifers. Four separate algae cultures were tested with variable concentrations of rotifers: 0, 25, 75, 200 per/mL. Counts of rotifer and algae populations (small and large), and turbidity were recorded in order to confirm selective grazing and evaluate settling improvement. pH, temperature, and ammonia concentrations were measured to determine how the microenvironment changed in response to varying rotifer populations. These water characteristic variables are critical to rotifer reproduction and survival. Therefore, under certain temperature, pH, and ammonia conditions, created by varying rotifer concentrations, selective grazing of rotifers on smaller algae (1-10 micrometers) will improve total algae settleability.

Methods

This study took place at the Algae Laboratory at the Richmond Field station. Water samples were taken from the Delhi Advanced Integrated Wastewater Pond System located in Central California. The pond has a 1.5 million gallon per day design flow and takes up 22 acres. The wastewater is from municipal sources and is pretreated for large algae using percolation beds (Lundquist 2000).

Four 500 mL beakers filled with Delhi pond water were continuously illuminated in incubation chambers at temperatures of 22-24 degrees Celsius. Added to each beaker was a different concentration of rotifers (Brachionus calyciflorus) (0, 25, 75, 200) with one beaker serving as a control (zero rotifers). Monitoring of algae and rotifer populations, pH, temperature, ammonia, and turbidity occurred at 0, 2, 4, and 7 days for each treatment. At the end of each 7 day trial, samples were replaced with fresh Delhi wastewater, which were taken on a weekly basis from the High Rate Pond. A total of ten replicates were performed.

The algae and rotifer concentrations was measured using a known volume of a pipette under a high-powered microscope following Copenhagen's methods, 1998. The rotifers were anesthesized with isopropyl alcohol to facilitate the counting. Dominant algae species were identified and categorized as small (1 to 10 micrometers) and large (greater than 10 micrometers).

Temperature and pH was measured in degrees Celsius using a Denver Instruments universal meter. To get the most precise pH values, manual standardization of pH in buffer solutions were performed. Ammonia concentrations were measured using LaMotte brand ammonia test kits. Turbidity was determined using a 6145 Klett meter, standardized with deionized water.

Results

Sample	Time (days)	Turbidity	Temp (C)	PH	Ammonia	Algae Cnt (sm)	Algae Ct (lg)	Rotifer	Change in Rotifer pop
Control	0	59.3	24.1	7.4	0.63	4500	2100	0	0
Control	2	53.5	24.1	8.3	1.63	4000	1400	0	0
Control	4	48.5	24.3	9.2	2.07	3700	1100	0	0
Control	7	46	23.9	9.3	2.22	3300	850	0	0
Rot-25	0	59.3	24.35	7.4	0.63	4500	2100	25	0
Rot-25	2	47	24.4	8.1	2.25	3600	1250	110	85
Rot-25	4	43.5	22	9	2.27	3000	1000	173	63
Rot-25	7	42.5	23.14	9.2	2.47	2900	975	311	138
Rot-75	0	59.3	21.4	7.4	0.63	4500	2100	75	0
Rot-75	2	41.2	22.4	8	2.2	3100	1450	275	200
Rot-75	4	31.2	22.2	8.3	2.37	2300	1100	580	305
Rot-75	7	29	23.4	9.4	4.47	1800	875	879	299
Rot-200	0	59.3	23.5	7.4	0.63	4500	2100	200	0
Rot-200	2	32.5	22.3	8.7	3.42	2300	1550	795	595
Rot-200	4	38.8	22.1	9.7	4.8	2600	1200	531	-264
Rot-200	7	43	23.5	9.7	6.12	3200	775	41	-490

Table 1: Averaged data by treatment

A total of ten replicates were performed for each treatment. Table 1 shows the averages of the replicates per treatment.

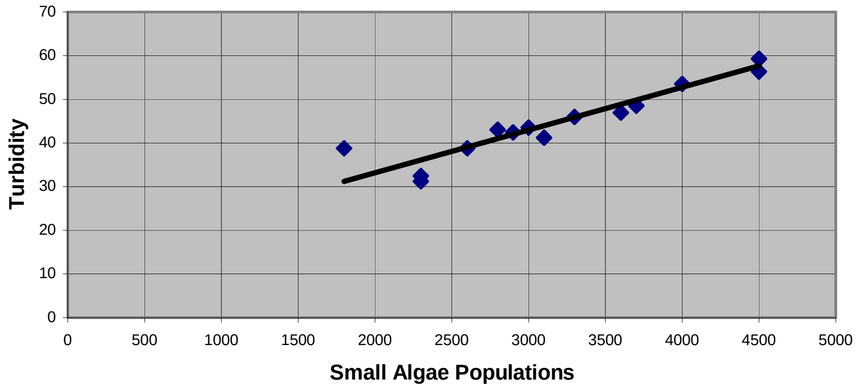

Figure 2: Turbidity vs. Small Algae Count

The dominant species of small algae were chlorella. There is a strong positive correlation between small algae counts and turbidity (r squared=0.84 and p=0.012) across all treatments (figure 2).

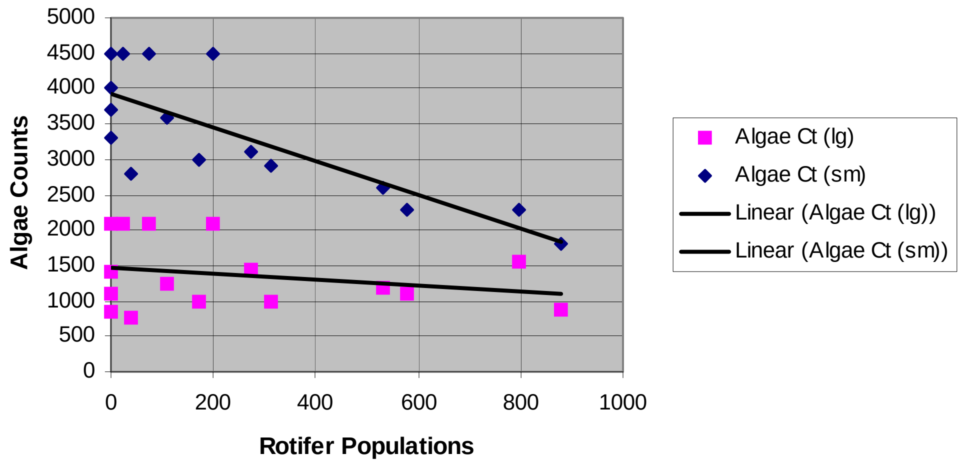

Figure 3: Algae Counts (small and large) vs. Rotifer Populations

Reduction in small algae populations was also highly correlated with increasing rotifer populations (r-squared=0.65). However, large algae populations were unaffected by increasing rotifer populations (r-squared=0.04) which confirms that selected grazing did indeed occur (figure 3). In addition, flocs of small algae cells lumped together were observed in treatments with rotifers.

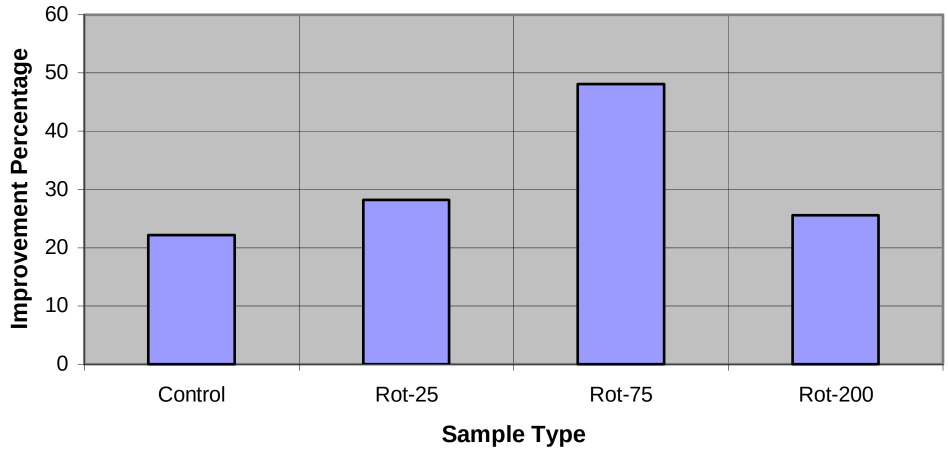

Figure 4: Improvement Percentage of different treatments after 7 days

In all treatments, improved settleability occured with time. Treatments with rotifers experienced significant settling improvement, by as much as 48% compared to day zero of the control and 27% compared to day seven of the control (figure 4). Increased rotifer populations also resulted in lowered turbidity, except for the 200 rotifer treatment. This sample experienced a significant reduction in turbidity from zero to two days. After two days, the turbidity began to increase (figure 5 and table 1). Regression analysis for the pooled data of total turbidity vs. total rotifer concentration yielded an R-squared value of 0.53 and a p-value of 0.023 which is statistically significant. This trend is also apparent in the small algae counts since small algae populations were positively correlated with turbidity (figure 1).

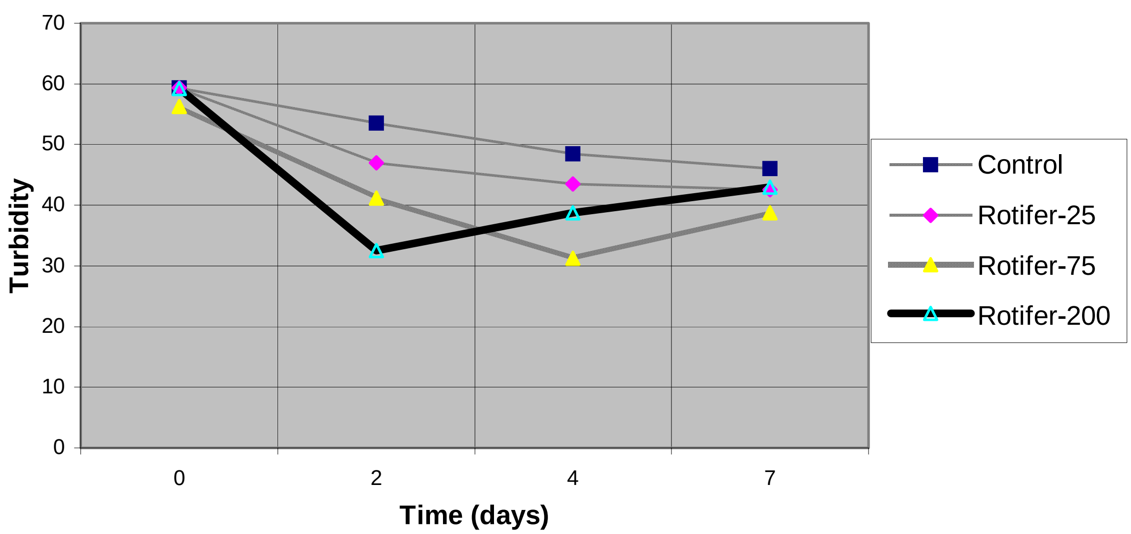

Figure 5: Relationship between turbidity and time for each treatment

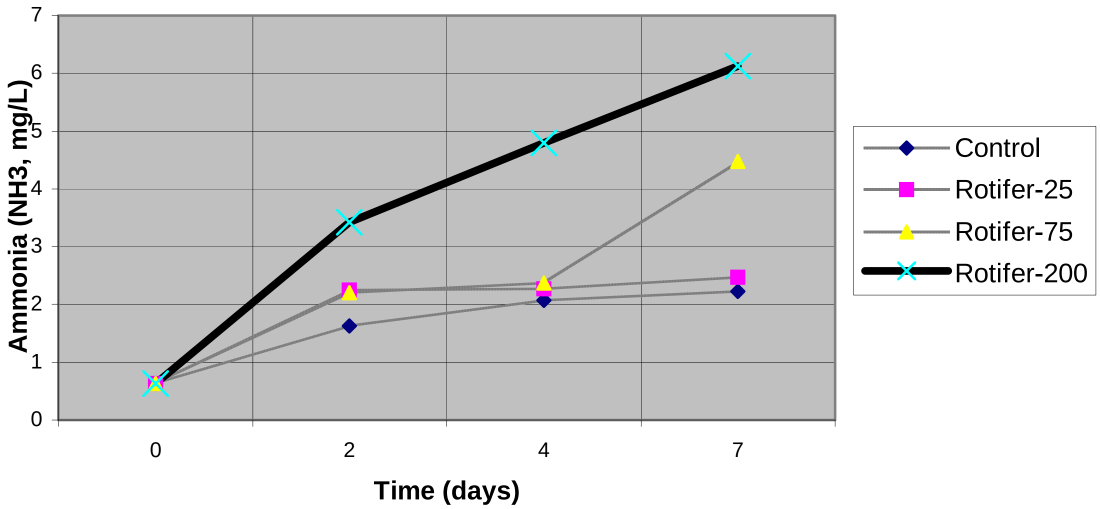

Figure 6: Relationship between ammonia and time or each treatment

Temperature remained steady between 22-25 degrees Celsius. pH ranged from 7.4 to 9.7, increasing with time (table 1). Regression analysis of pH vs rotifer concentration shows no significance despite a positive correlation between the two variables (r-squared=0.49 p=0.18). Ammonia concentrations ranged from 0.63 mg/L to 6.1 mg/L increasing with time and increasing with each treatment (figure 6). The highest ammonia concentration occurred for the treatment of 200 rotifers (initial population) after seven days (figure 6). At two days, the rotifer population for this treatment nearly quadrupled while after seven days, the population decreased to 41 rotifers, less than 25 percent of the initial population (Table 1).

Discussion

The results reveal several key findings. Small algae counts ran parallel to turbidity (figure 1). Also, populations of small algae (predominantly Chlorella) were significantly reduced with the presence of rotifers while counts of larger algae were unaffected (figure 2). This reduction in small algae is almost linear. In addition, increased flocs of algae cells were observed in treatments containing rotifers. From this, we can infer that rotifers selectively grazed on the smaller algae. Previous studies by Hansen (1997) also showed selective grazing of small algae by rotifers.

Settling improvement (change in turbidity) occurred over time in the control as well as in samples with rotifers. The addition of rotifers accelerated settling by up to 48 percent (for treatment of 75 rotifers) compared to the control at zero days and 27 percent after seven days (Figure 3). Looking at table 1 and figure 3 for the treatment of 200 rotifers, the large initial population of rotifers did not result in a large reduction in small algae after seven days. The rotifer population in this treatment quadrupled at two days, and at seven days decreased to less than 25% of its initial population (table 1). The trend in turbidity for this treatment (figure 4) and counts of small algae (table 1) follow a similar pattern. At two days, turbidity and small algae counts were reduced by 50 percent, however after two days, these variables began to increase. This finding suggests that the large initial population of rotifers altered the microenvironment specifically small algae populations, pH, and ammonia.

Small algae, the rotifer's source of food is severely reduced with increasing rotifer populations (figure 2). This reduction in small algae increases rotifer competition for nutrients therefore ultimately reducing rotifer reproduction and survival. A comparison of two treatments shows the density dependence. Initial treatments of 75 rotifers and treatment of 200 rotifers showed that a population of 2300 small algae allowed for a rotifer population of 570 to grow while a population of 879 was reduced (table 1). Therefore overgrazing occurred in populations greater than 531 rotifers. In the initial treatment of 75 rotifers, the experiment was not carried on beyond 7 days therefore the carrying capacity of the system could not be determined.

In addition to serving as a source of nutrients to rotifers, algae play an important role in the high rate pond by fixing ammonia that is produced by the bacteria to produce oxygen (figure 1). Algae also reduce dissolved carbon dioxide concentrations through photosynthesis which raises the pH level. Ammonia and pH are related by the following equilibrium relationship:

$$NH_3 + H_2O \Leftrightarrow NH_4 + OH^-$$

A high pH would move the equilibrium to the left and raise the ammonia concentration. Thus, a reduction of algae would result in increased levels of ammonia. Previous studies of threshold concentrations of ammonia show that ammonia concentrations of 3 mg/L impair rotifer reproduction. Concentrations above 5 mg/L are lethal and rotifers die within two days (Schluter 1996). The rotifers also excrete ammonia, which further increases the ammonia concentration. This explains the increased ammonia concentrations over time in all treatments and why the treatment of 200 rotifers experienced a high ammonia concentration (6.1 mg/L) which resulted in a mass rotifer die off after four days (figure 6). This reduction in the rotifer population reduces its capacity to graze on small algae populations which explains why small algae populations and turbidity began to increase after four days for that treatment.

The results indicate that rotifers selectively grazed on smaller algae. This suggests that rotifers are a potential solution to improvement of algae settleability in high rate ponds. However, the results also indicate that improvement is limited due to the rapid reproduction of rotifers. Large populations of rotifers result in overgrazing of algae populations which alters the water characteristics, primarily the ammonia levels which have an adverse effect on rotifer reproduction and survival.

The results presented are only a qualitative analysis of the effect of rotifers on algae settleability through selective grazing. It does not attempt to make any quantitative assertions as to the exact number of rotifers required due to the limited sample size and poor study design. Predation on rotifers by macrozooplankton, Daphnia, was not studied and could have been a confounding variable that reduced rotifer populations. Though temperature remained steady, ammonia and pH were uncontrolled for and changed with different rotifer concentrations. In addition, the results showed density dependence in the rotifer population, however it is unclear what the carrying capacity of the environment is for the rotifers. This is important to determine the threshold population that causes overgrazing on algae. Also, the effect of continuous lighting on the system increased the photosynthetic capacity of the algae. This increased pH and caused ammonia levels to rise above those naturally found in the treatment ponds. Therefore, a study using controlled ammonia and normal daylighting conditions is required to precisely determine the impact of these variables on rotifer survival and their ability to improve algae settleability.

Acknowledgements

I'd like to personally thank the staff at the Richmond Field Station Algae Lab, Tryg Lundquist, Ingrid Zubieta, Glen Anderson, and Professor Oswald for all their help with the study design and methodology. Thanks to my thesis advisor, Justin Victor Remais and all my senior thesis classmatesfor their patience and support. A special thanks to my family for all their love and encouragement.

References

Alvarez-Cohen, Lisa and Nazaroff, William. 2000. *Environmental Engineering Science*. 2nd Edition. J. Wiley and Sons, New York. Chapter 7.

Canovas, S. and Picot, B. Seasonal development of phytoplankton and zooplankton in a high rate algal pond, 33 (7), 199-206.

Cromar, N.J. and Fallowfield, H.J. 1996. Influence of environmental parameters on biomass production and nutrient removal in a high rate algal pond operated by continuous culture. *Water Science and Technology*, 34 (11), 133-139.

Cronberg, Gertrud and Hansson, Lars-Anders. 1998 Size structure and succession in phytoplankton communities: the impact of interactions between herbivory and predation. *Oikos*. 81, 337-345.

Esen, I. and Puskas, K. 1994. Production and separation of algae in a high rate ponds system. *Environment International*, 20 (4), 541-549.

Esen, I. and Puskas, K. 1991. Algae removal by sand filtration and reuse of filter material. *Waste Management*. 11, 59-65.

Gleick, P.H. 1998. The world's water: 1998-1999. Island Press, Washington D.C. Hansen, Benni and Louise Wittrup, Thomas Wernberg-Moller. 1997. Particle grazing efficiency and specific growth efficiency of the rotifer Brachionus plicatilis. *Journal of Experimental Marine Biology and Ecology*. 215, 217-233.

Hoff, Frank. 1987. *Plankton Culture Manual.* Florida Aquafarms, Florida

Kirk, Kevin. 1997. Life-history responses to variable environments: starvation and reproduction in planktonic rotifers. *Ecology*. 78 (2), 434-441.

Kirk, Kevin and Merriman, Jodie. 2000. Temporal patterns of resource limitations in natural populations of rotifers. *Ecology*. 81 (1), 141-149

Oswald, W.J. 1988. *Micro-algae and waste-water treatment*. Cambridge University Press, New York. 305-354

Rovirosa, N. and Sanchez, E. 1996. An integrated system for agricultural wastewater system. *Water Science and Technology*, 32 (1), 165-171.

Schluter, M. and Groenberg, J. 1985. The inhibition by ammonia of population growth of the rotifer, brachionus rubens, in continuous culture. *Acquaculture*, 46, 215-220.

Hall, Thomas. 1989. Bioflocculating high rate algal ponds: control and implementation of an innovative wastewater treatment technology./

Nurdogan, Yakup .1988. Microalgal separation from high rate ponds.

USEPA. 1998. *Clean Water Act Overview*. www.epa.gov/owow/cwa.history.htm