# Algal Concentration and Species Composition in Experimental Maturation Ponds with Effects of Aeration and Recirculation

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#### Abstract

Three experimental maturation pond systems receiving humus tank effluent from a conventional sewage treatment works (Daspoort, Pretoria) were studied over a twelve month period. The effects of aeration and recirculation of pond water on the algal concentration were investigated in comparison with no agitation of pond water. The algal composition of the systems was studied with emphasis on the non-agitated (control) system.

The algal concentrations of all three systems exhibited wax and wane patterns. An Algal Concentration Index (ACI) was developed as a means of comparing the different algal concentration patterns of the three systems. The aerated system appeared to be the most efficient in terms of maintaining a high algal concentration. Algal concentration declines occurred during both summer and winter months in the recirculated and non-agitated systems but only during the summer in the aerated system. Possible reasons for the algal concentration pulses and subsequent declines are discussed.

Members of the Chlorophyceae were overall dominant in the pond systems while species of the Chlorophyceae, Euglenophyceae, Cryptophyceae and Cyanophyceae were dominant during different algal pulse periods. A seasonal algal succession was noted for the non-agitated system and the species composition was different for the three systems.

# Introduction

The importance of sewage maturation ponds was discussed in a previous paper (Shillinglaw and Pieterse, 1977) in terms of their ability to remove plant nutrients during periods of high algal concentration. Physical, chemical and biological parameters of one experimental maturation pond system (system A, Fig. 1) at the Daspoort Sewage Works, Pretoria, were discussed and the possible causes of algal population declines mentioned (Shillinglaw and Pieterse, 1977).

Previous research on maturation ponds (Keirn, 1974, 1975) indicated that aeration of maturation pond water prevented drastic declines in the algal concentration. It was not clear from Keirn's research whether it was aeration or merely agitation of pond water which was the important feature in preventing algal population declines. Hence this study investigates the effects of aeration (with compressed air) and

agitation (in the form of recirculation of water) on algal concentration, to see which system could maintain the highest algal concentration.

In the study of the algal species composition, a detailed investigation was carried out only for the control system (no agitation of water), especially just before, during and after the periods of peak algal concentrations as indicated by the chlorophyll a concentration. These observations were made because research by Keirn (1975) indicated that only members of the Chlorophyceae were dominant during algal concentration declines. Keirn suggested that any growth suppressing factor causing declines was probably effective mainly against green (Chlorophycean) algae.

# Materials and Methods

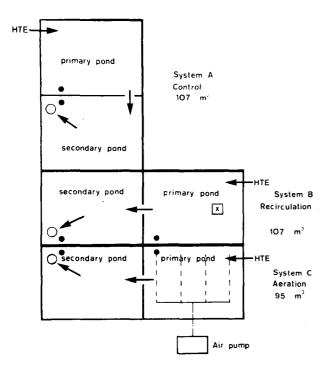
Six experimental maturation ponds at the Daspoort Sewage Works (Pretoria) were used. They were arranged into three systems, each consisting of a primary and secondary pond in series. The ponds were all 1 m deep and lined with concrete. System A (Fig. 1) was operated as the control, and had a total volume of 107 m3; the water in this system was not aerated or artificially agitated in any way. This system is the same system described in a previous paper by Shillinglaw and Pieterse (1977). System B also had a total volume of 107 m3, but the water in the primary pond was agitated by a recirculation pump giving a probable mixing rate of one turnover per hour. System C had a total volume of 95 m<sup>3</sup> and the primary pond was aerated with compressed air from an air pump blowing through many small holes in four 10 cm diameter plastic pipes placed on the bottom of the pond. The capacity of the air pump was 0,3 m<sup>3</sup> min<sup>-1</sup> at 1 m (lepth giving a probable mixing rate as estimated by bubble size and bubbling rate, of one turnover per hour (Keirn, 1975). Agitation by the recirculation pump would result in a certain amount of aeration of the recirculation systems' water, but this would probably be insignificant compared to the aerated system. The aeration of compressed air in system C would have increased the concentration of carbon dioxide in the water.

All three systems received humus tank effluent (HTE) as their influent water. The retention period for each system was approximately 5 days.

The sampling procedure and period was the same as

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- x Recirculation pump
- Sampling position
- Outflow funnel

Figure 1
Diagram of the three experimental pond systems (A, B and C).

described previously by Shillinglaw and Pieterse (1977). Samples were taken between 14h00 and 15h00. From August 1975 to February 1976 the ponds were sampled five times a week while samples were collected twice a week for the remainder of the study period. The chlorophyll a concentrations of water samples taken with a hose-pipe sampler (18 mm internal diameter) were determined by the method described by Marker (1972) using 95 per cent methanol as the extraction solvent. The hose-pipe samples were taken from the surface to approximately 60 mm from the bottom.

The algal species present were determined from hose-pipe water samples fixed with a 2 per cent acid Lugol's solution (Vollenweider, 1969). Algal cell counting was done according to the method of Utermöhl (1958).

Quantitative microscopial algal counts were made only on samples collected from the control system (system A). A detailed comparison of the algal populations of all three systems is therefore not possible. Gross examination of the algal samples from systems B and C were performed to ascertain superficially the dominant algal species present in the ponds before, during and after the algal population pulses. This examination was made with relative ease as in all samples examined, one or at the most two species of algae were outstandingly dominant.

## Results

## Chlorophyll a concentration

For the purpose of this study the chlorophyll a concentration of the pond water was taken to represent a quantitative estimation of the algal concentration.

The results (Fig. 2) for the three systems clearly showed periods of sudden increases in algal concentration to high concentrations followed rapidly by periods of sudden decreases. For the purpose of this study these periods were called 'pulses', being arbitrarily considered as any algal concentration which was two or more times as great as the mean annual algal concentration (Pennak, 1946). On most occasions these algal pulses occurred simultaneously in both ponds of a system.

The combined mean algal concentration for primary and secondary ponds for the twelve months of study for systems A, B and C were 160, 174 and 272  $\mu$ g chlorophyll a per litre respectively. Systems A, B and C had 10, 6 and 4 algal pulses respectively during the study period (Fig. 2). It follows, therefore, that the control system experienced the greatest number of algal pulses (and declines) and the lowest combined mean algal concentration while the aerated system experienced the least amount of algal pulses and the highest combined mean algal concentration.

The algal pulses indicated by the chlorophyll a concentration (Fig. 2) are summarized in Table 1. Evidently for system A the more intensive algal pulses occurred in the secondary pond during the period from August to October 1975, while during the period November 1975 to July 1976 the most intensive pulses occurred in the primary pond. The pulses in February and March were of almost equal intensity in the primary and secondary ponds. In system B the pulses were always more intensive in the secondary pond. System C exhibited confusing results as the more intensive pulses occurred in the primary pond on two occasions, while the secondary pond had an outstandingly intensive pulse during late September. The pulse in early September was of almost equal intensity in the primary and secondary ponds.

In comparing the concentration of algal biomass in the three systems, it was necessary to consider the length of time during which pulses existed, the mean algal concentration and the average peak algal concentration during pulses. For this reason the combined (for two ponds) total duration of all pulses for each system was calculated as a percentage of the study period (D in Table 2). This term (D) was calculated by measuring the pulse periods of both ponds on the reference line (twice the combined mean algal concentration) of the chlorophyll a concentration (Fig. 2) and relating it on a percentage basis to the twelve month study period. The per cent duration of pulses, mean algal concentration and average peak algal concentration during pulses were used to calculate an Algal Concentration Index (ACI) for each system in the following manner:

$$ACI = 2x \frac{P.D.}{2} \qquad (1)$$

where:

2x = Twice the combined mean chlorophyll a concentration for the twelve months of study for the system.

P = Average peak chlorophyll a concentration above the 2x reference line for the twelve months of study.

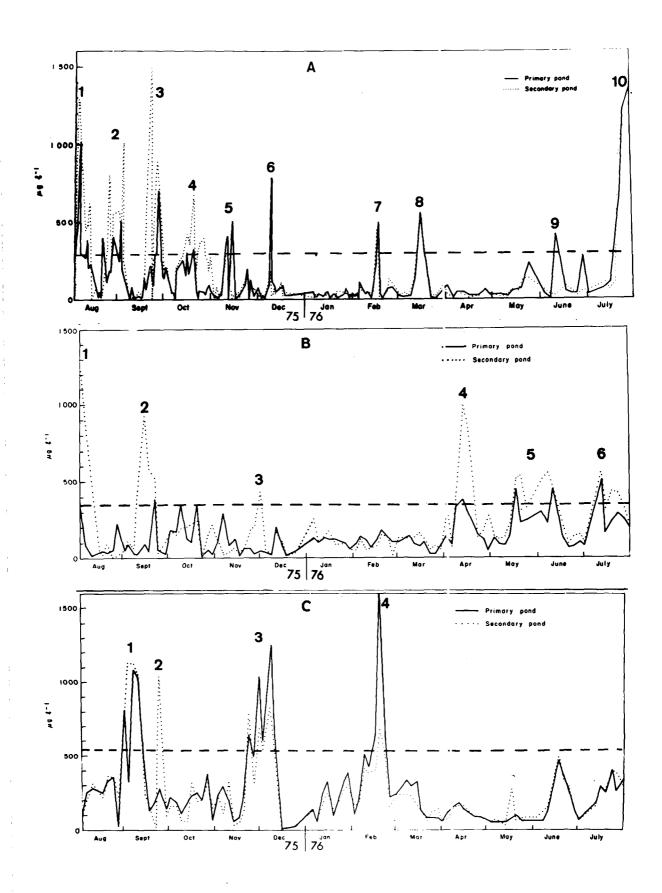


Figure 2
Chlorophyll a concentration (in µg (°)) for systems A, B and C. The horizontal broken line represents twice the combined mean (lgal concentration for the twelve months of study. The numbers (1 to 10) indicate the chlorophyll a concentration pulses (A: slightly modi, ied from Shillinglaw and Pieterse, 1977).

TABLE 1
TABLE SUMMARIZING THE OCCURRENCE OF
ALGAL PULSES IN THE EXPERIMENTAL MATURATION POND SYSTEMS. THE POND IN WHICH THE
PULSE WAS MOST SIGNIFICANT IS IN ITALICS

System and Treatment	Number of pulses	Pulse Number	Pond(s) of occurrence	Month(s) of Occurence
A Control	10	1	Primary & Secondary	August
		2	Primary & Secondary	August & September
		3	Primary & Secondary	September
		4	Primary & Secondary	October
		5	Primary & Secondary	November
		6	Primary	December
		7	Primary & Secondary	February
		8	Primary & Secondary	March
		9	Primary	June
		10	Primary & Secondary	July
B Recircu-	6	1	Primary & Secondary	August
lation		2	Primary & Secondary	September
		3	Secondary	December
		4	Primary & Secondary	April
		5	Primary & Secondary	May & June
		6	Primary & Secondary	July
C Aeration	4	1	Primary & Secondary	September
}		2	Secondary	September
		3	Primary & Secondary	November & December
		4	Primary & Secondary	February

Note: 2x, P and D are combined values for the primary and secondary ponds of each system.

TABLE 2
ALGAL CONCENTRATION INDICES (ACI) FOR THE
THREE SYSTEMS

System	D (per cent)	<b>P</b> (μg ( <sup>-1</sup> )	2x̄ (μg ( <sup>-1</sup> )	ACI
Α	15	388	320	9,3 x 10 <sup>5</sup> 5,8 x 10 <sup>5</sup>
В	14	240	348	$5.8 \times 10^{5}$
С	I 1	416	544	12,4 x 10 <sup>5</sup>

#### Note:

- 1. The ACI values were calculated by using equation (1)
- 2. D = Total per cent duration of the pulses for the twelve months of study
- 3.  $P = Average peak chlorophyll \alpha concentration above the <math>2\overline{x}$  reference line for the twelve months of study
- 4.  $2\overline{x}$  = Twice the combined mean chlorophyll *a* concentration for the twewlve months of study

The ACI values (Table 2) indicated that the overall growth conditions in the systems were most unfavourable (lowest ACI value) in system B while system C had the highest ACI value, and system A a value mid-way between that of systems B and C.

## Microscopical algal counts

The microscopical counts of the total number of algal cells per unit volume of water and the percentage composition of the most dominant algal species for system A (control) are presented in Fig. 3. Percentage composition was calculated as follows: The cell count for each algal species was calculated as a percentage of the total cell count for a particular sample. These percentage compositions for each algal species were plotted in Fig. 3 on a linear scale with the total cell count for a particular sample taken as 100 per cent. Therefore the 100 per cent value in Fig. 3 was different for each sample as the total cell count was different. This is merely a visual way of presenting total cell counts and percentage composition on the same figure. Moreover the species composition cannot be read as a direct cell count on the ordinate axis as this is a logarithmic scale.

The total algal cell counts (Fig. 3) followed similar, but not identical, trends to those of the chlorophyll a concentration (Fig. 2) for system A. For convenience the algal cell counts and composition will be considered in terms of the ten algal pulses described for the chlorophyll a concentration results. The algal cell counts and composition for the primary and secondary ponds of system A were similar, hence the two ponds are considered collectively in this presentation of the algal cell count results with exceptions being noted.

## Pulse 1

This pulse occurred at the beginning of August with Chlamydomonas globosa Snow being the dominant (up to 65 per cent) algal species in both ponds. Cryptomonas ovata Ehrenberg was also prevalent during this period (about 20 per cent).

D = Total per cent duration of the pulses for the twelve months of study.

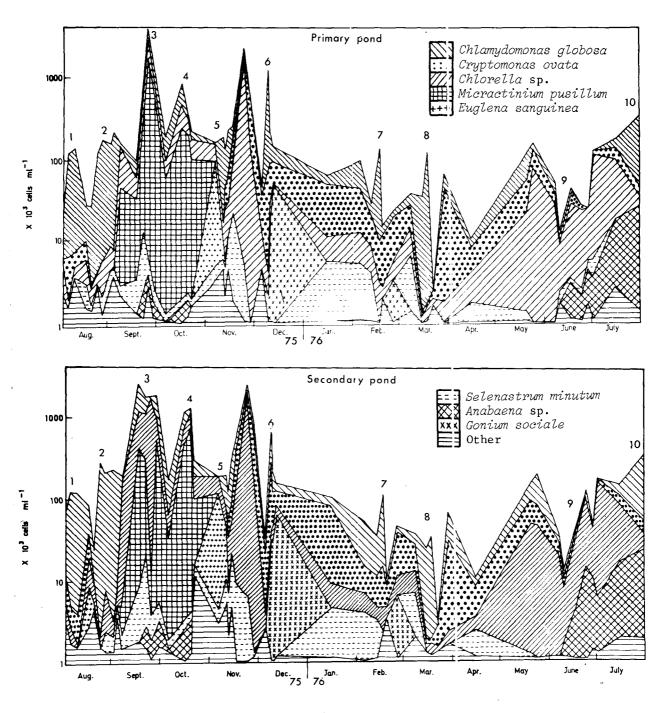


Figure 3

Total algal cell counts (in cells me<sup>1</sup>) and percentage composition of the dominant algal species for the primary and secondary ponds of system A. The numbers (1 to 10) represent the chlorophyll a concentration pulses (see Fig. 2).

# Pulse 2

Chlamydomonas globosa remained the dominant species during August and was again the most important species involved in pulse 2 at the end of August, early September (as high as 87 per cent in the secondary pond). During the decline phase of the

pulse (Fig. 3) C. globesa lost its dominance and was a small component of the algal population (below 5 per cent) towards the end of the decline. Micractinium pusillum Fresenius became the dominant species (a pout 50 per cent) while Chlorella sp. and Selenastrum minutum (Naeg) Collins both became important (about 20 per cent each) during this period.

#### Pulse 3

This pulse occurred towards the end of September and resulted in a large concentration of algal cells (over 5 million cells per ml) which is in agreement with the high chlorophyll a concentration (Fig. 2). Micractinium pusillum was the dominant species (75 per cent) in the primary pond during this pulse. In the secondary pond the third pulse in the chlorophyll a concentration (Fig. 2) was divided into two peaks. From the algal composition results (Fig. 3) it appears that Chlorella sp. (65 per cent) and Selenastrum minutum (27 per cent) were the dominant species during the first peak while Micractinium pusillum (60 per cent) was dominant during the second peak.

#### Pulse 4

M. pusillum remained the dominant in both ponds (above 50 per cent) during October and was 75 per cent dominant during the fourth pulse which occurred during the second half of October. M. pusillum was completely absent by the end of October. Anabaena sp appeared during this pulse and comprised 12 per cent of the algal population in the secondary pond. It had, however, completely disappeared by the end of this pulse.

#### Pulse 5

The fifth pulse in the chlorophyll a concentration results (Fig. 2) did not appear as a pulse in the algal cell counts (Fig. 3). The dominant algal species during this period (first half of November) was Euglena sanguinea Ehrenberg (62 per cent). E. sanguinea was only dominant for a brief period and was absent by the end of this pulse period.

The algal cell count results (Fig. 3) indicate a peak towards the end of November. This algal peak is represented by a small chlorophyll a concentration peak (Fig. 2) but not by a pulse. The dominant algal species during this peak period was the very small unicellular algae, Chlorella sp. (about 75 per cent).

# Pulse 6

Co-dominance between Cryptomonas ovata (39 per cent) and Chlamydomonas globosa (39 per cent) existed in the primary pond during this pulse in the first half of December. The secondary pond had co-dominance between Cryptomonas ovata (35 per cent) and Gonium sociale (Duj) Warming (42 per cent). G. sociale became the dominant species (82 per cent) in both ponds immediately after this pulse period.

# Pulse 7

Cryptomonas ovata (48 per cent) and Selenastrum minutum (43 per cent) were the dominant species during January. A dense population of the filamentous alga, Mougeotia sp. occurred on the surface water of the ponds during January. This algal population did not cause any notable increase in the chlorophyll a or algal cell count results which was probably due to the method of sampling as discussed in Shillinglaw and Pieterse (1977). During the early part of February, Chlamydomonas globosa gained dominance and was about 50 per cent of the algal population during the seventh pulse which occurred in mid-February. Cryptomonas ovata (30 per cent) and Chlorella sp. (about 20 per cent) were also important during this pulse. Chlamydomonas globosa lost its dominance rapidly during the decline

phase of this pulse while Cryptomonas ovata (about 35 per cent) and Gonium sociale (50 per cent) became dominant after the pulse. Selenastrum minutum (43 per cent) also became dominant for a brief period in early March.

## Pulse 8

Chlamydomonas globosa regained its dominance briefly during mid-March and was dominant (90 per cent) during the eighth pulse. Again the cell concentration of C. globosa was reduced (below 10 per cent of the algal composition) during the decline phase of this pulse.

Cryptomonas ovata (37 per cent) became the dominant species during April while Chlorella sp. became dominant (87 per cent) during May, producing a cell concentration peak towards the end of May. This peak did not show up as a pulse in the chlorophyll a concentration results (Fig. 2).

## Pulse 9

According to the chlorophyll  $\alpha$  concentration results (Fig. 2) a pulse was observed during mid-June in the primary pond and not in the secondary pond. The algal cell count results (Fig. 3) indicate that a peak occurred slightly later in the primary pond, while in the secondary pond the algal cell count was increasing at the time of the chlorophyll a concentration pulse in the primary pond. The algal cell count in the secondary pond continued to increase and peaked towards the end of June at a higher count than the peak in the primary pond. Chlorella sp. and Anabaena sp. (both about 50 per cent in the secondary pond) were co-dominant during this pulse.

# Pulse 10

Anabaena sp. was dominant (45 per cent) during the last pulse at the end of July. Chlamydomonas globosa concentration started to increase once again during this pulse (27 per cent).

Detailed observations on the algal cell concentration and composition were not made for systems B and C. However, the algal cell samples collected during periods of noted chlorophyll a concentration pulses in systems B and C (Fig. 2) were examined microscopically to ascertain the dominant algal species during pulse periods. These qualitative observations for systems B and C plus a summary of the qualitative results for system A are presented in Table 3.

Members of the Chlorophyceae (Table 3) were most frequently dominant during algal cell concentration pulses in all three pond systems. The list of dominant algal species during the pulses indicates that many of the species that occurred in system A (control) also occurred in systems B and C. Only a few dominant species were unique to systems B and C (e.g. Eudorina elegans Ehrenberg, Pandorina morum Müll.) Bory., Euglena deses Ehrenberg, Scenedesmus quadricauda (Turp.) de Brebisson and Actinastrum hantzschii Lagerheim.

# Discussion

# Chlorophyll a concentration

The chlorophyll a concentration (Fig. 2) exhibited a general pattern of wax and wane of the algal populations in all the pond systems. This wax and wane pattern is indicative of algal popu-

#### TABLE 3

LIST OF THE DOMINANT ALGAL SPECIES IN THE THREE SYSTEMS DURING THE VARIOUS NOTED ALGAL CONCENTRATION PULSES (AS INDICATED BY THE CHLOROPHYLL a CONCENTRATION) (SYSTEM A FROM SHILLINGLAW AND PIETERSE, 1977)

System	Pulse Number	Month(s) of	Dominant algal species
		Occurrence	
A	1	August	Chlamydomonas globosa Snow
	2	Aug. & Sept.	Chlamydomonas globosa Snow
	3	September	Micractinium pusillum Fresenius
	4	October	Micractinium pusillum Fresenius
	5	November	Euglena sanguinea Ehrenberg
	6	December	Cryptomonas ovata Ehrenberg Chlamydomonas globosa
			Snow
	7	February	Chlamydomonas globosa Snow
	. 8	March	Chlamydomonas globosa Snow
	9	June	Chlorella sp. & Anabaena sp.
	10	July	Anabaena sp. & Chlamydomonas globosa Snow
В	1	August	Euglena sanguinea Ehrenberg Cryptomonas ovata Ehrenberg
	2	September	Euglena sanguinea Ehrenberg
	3	December	Chlamydomonas globosa Snow
	4	April	Pandorina morum (Müll) Euglena deses Ehrenberg Scenedesmus quadricauda (Turp.) de Brebisson
	5	May &	Chlamydomonas globosa Snow
	6	June July	Chlamydomonas globosa Snow
С	1	September	Actinastrum hantzschii Lagerheim Micractinium pusillum Fresenius
	2	September	Chlamydomonas globosa Snow
	3	Nov. & Dec.	Eudorina elegans Ehrenberg
	4	February	Chlamydomonas globosa Snow

lations subjected to changing environmental conditions. All the pond systems had the potential to support a large algal concencration, but this concentration could only be sustained for brief periods (pulse periods). All the noted chlorophyll a concentration pulse periods were followed by declines to relatively low concentrations. The development of chlorophyll a concentration pulses were basically synchronized for both ponds of a system. However, the pulses usually resulted in a higher chlorophyll a concentration in one of the two ponds of a system during most of the observed pulses (see Table 1 and Fig. 2). System B (recirculation) was the only system which exhibited a consistently higher chlorophyll a concentration in the secondary pond during all the noted pulse periods (Fig. 2), possibly indicating a prevention by circulation (in primary pond) of conditions resulting in pulses. In systems A (control) and C (aeration) the highest chlorophyll a concentration during pulse periods occurred in the primary ponds on some occasions and in the secondary ponds on others (Fig. 2). Thus high chlorophyll a concentrations were not confined to one particular pond of either system. The separation of the systems into primary and secondary ponds did not influence the size of the algal population that could be obtained in a system. It is possible that a single pond with the same volume as the experimental pond system used in this study, would have produced similar sized algal concentrations and also followed similar wax and wane patterns as found in this study. Sless (1974) also suggested that the same effects would be achieved in a pond system consisting of a single pond of two to three times the surface area of individual ponds, with a correspondingly longer retention time (per individual pond).

The chlorophyll a concentration trends for the three systems were different (Fig. 2). System A exhibited the largest number of chlorophyll a concentration pulses of all the systems (Table 1), indicating that the environmental conditions necessary for pulses occurred most often in system A. Conditions necessary for large algal populations were therefore not maintained for long periods. Lack of mixing could have made system A less homogenous than the other ponds and therefore more susceptible to changes. In system B a different trend emerged where less pulses occurred, but each pulse existed for a longer duration than in system A (see Fig. 2). This indicates that the conditions necessary for pulses occurred less frequently in system B, but were maintained for longer durations. When the total duration of algal pulse periods were calculated as a percentage of the total study period, systems A and B gave similar values (D in Table 2). Also, the mean chlorophyll a concentration for the study period in systems A and B were similar (160 and 174  $\mu$ g  $\ell^{-1}$ respectively). All this evidence suggests that the algal concentration of systems A and B were similar and it would be very difficult to conclude that one system operated more efficiently than another in terms of the system's ability to produce and maintain a high algal concentration.

System C exhibited a markedly different pattern compared to the other two systems. In system C very few pulses occurred, and the total duration of these pulses (D in Table 2) was less than the total durations found in systems A and B. This indicated that the environmental conditions necessary for algal pulses occurred less frequently in system C than in the other two systems, and the favor rable conditions for pulses were not maintained for long periods in system C. This suggests that system C was the most inefficient system in terms of algal pulse frequency and duration. However, the mean chlorophyll a concentration for system C was almost double the values for the other two systems. This indicated that, although less algal concentration pul-

ses occurred in system C, the algal concentration between pulses was higher in system C than in systems A and B (see Fig. 2). Also, when the maximum algal concentrations produced during algal pulses were averaged for the three systems, A and C had similar values higher than that of B. Therefore, when the ACI (Algal Concentration Index) was calculated (Table 2), it became evident that the ACI values of the three systems were different. From this it can be concluded that system B was least efficient in terms of producing high algal concentrations for the entire study period, and system C was the most efficient, while system A had an efficiency midway between systems B and C. Therefore, it follows that the conditions in system C were the most favourable for algal growth. This evidence also suggests that aeration with compressed air produced the favourable conditions for algal growth in system C. The favourable conditions were not produced merely by agitating the water, as the water recirculation system (B) produced the lowest ACI value. Possibly the favourable condition in the aerated system was an increase in carbon dioxide. The ACI values over-emphasize extreme conditions in the systems but this is advantageous as the importance of studying maturation pond functioning is to attempt to understand the cause and effects of extreme conditions which occur resulting in severe algal concentration declines.

The chlorophyll a concentration pulses occurred during both summer and winter months of the study period in systems A and B, but only occurred during summer months in system C. Van der Post and Toerien (1974) indicated in their work with the maturation ponds at Windhoek, that algal population declines to low concentrations occurred only during the warmer months of the year.

All the evidence of the wax and wane patterns exhibited by the chlorophyll a concentration of the three systems points to the intermittent presence of some factor which suppressed algal growth and/or removed algal cells from the system at a rapid rate. Another possibility is that an algal growth suppressor was almost continuously present, and only when the suppressing factors were intermittently absent, did the algal concentrations exhibit a pulse.

Another way in which the wax and wane pattern of the chlorophyll a concentration for the systems could be explained. is that the environmental conditions became favourable to algal growth which stimulated the algae to produce the pulse. Then possibly the decline phases of the pulses were caused either by some nutrient becoming limiting to algal growth at the peak algal concentration of the pulse, or else an inhibitory substance might have been produced by the algae at peak pulse conditions. If the decline phase of the pulse were due to an inhibitor, then the inhibitory substance must disappear during the decline phase thus allowing the algal population to reach pulse concentrations again.

Because of the fact that all three systems received the same inflow (HTE), and the algal pulses and subsequent declines were not synchronized in the systems it can be assumed that the inhibiting condition did not originate in the inflowing water. Inhibition, on this assumption, could then only have developed in the ponds themselves.

It must be emphasized that from the results it is impossible to suggest which of the above-mentioned causes of wax and wane patterns in the algal concentration might be applicable. It does seem, however, that nutrient limitation (except possibly carbon) does not play an important role as suggested by Keirn (1974, 1975). It can only be concluded that the overall conditions for efficient algal growth was most favourable in system C which indicates that aeration was able to reduce an inhibitory

effect, should such an effect exist, on algal growth in the system.

# Microscopical algal counts

When the algal cell count results (Fig. 3) are compared with the chlorophyll a concentration results for the control system (Fig. 2), it is obvious that these two parameters are visually correlated, and most of the observed chlorophyll a concentration pulses have corresponding peaks in the algal cell counts. On a few occasions there were discrepancies between the algal counts and chlorophyll a concentration. One of these occasions occurred during early November (pulse 5) where the chlorophyll  $\alpha$  concentration showed a pulse while algal cell counts did not show a corresponding peak. The dominant algal species during this period was Euglena sanguinea. One possible explanation for the discrepancy in the algal cell count and chlorophyll a concentration results, is that E. sanguinea cells are large thus possibly containing large amounts of chlorophyll a per cell which could have increased the extracted chlorophyll  $\alpha$  concentration markedly. The actual number of E. sanguinea cells was not high, thus giving a relatively low algal cell count. If the volume of the counted algal cells had been taken into account, then possibly the total counted algal cell volume during this pulse period would have shown a peak corresponding to the chlorophyll a concentration

On two other occasions (late November and late May) the algal cell counts exhibited peaks which did not correspond to pulses in the chlorophyll a concentration. During both of these occasions the dominant algal species was a very small-celled Chlorella sp. It was observed that these small algal cells passed through the glass fibre filters used in the chlorophyll a extraction method, and these cells were therefore not included in the chlorophyll a extracts of the water samples. Thus the chlorophyll a concentration results would not exhibit a pulse during these periods of Chlorella sp. dominance. Another discrepancy existed during pulse 9 of the chlorophyll a concentration results. Here the peak in the algal cell count for the primary pond occurred slightly later than the peak in the chlorophyll a concentration. Also, a peak occurred in the secondary pond algal counts after the chlorophyll a pulse (towards the end of June) which did not show up in the chlorophyll a concentration. These discrepancies are difficult to explain but again Chlorella sp. was one of the dominants during this period, thus possibly influencing the results as explained above.

From the description of the algal composition during the various algal pulses in system A, it is obvious that while members of the Chlorophyceae were involved in most of the algal pulses, members of the Cryptophyceae, Euglenophyceae and Cyanophyceae were also occasionally involved. The most frequently dominant algae in system A were unicellular forms of the Chlorophyceae. In systems B and C, other members of the Chlorophyceae (especially colonial forms) were frequently dominant during the algal pulses. This dominance of Chlorophyceae in sewage ponds has been noted by other workers, i.e. Patil et al. (1975), De Noyelles (1967) and Keirn (1974; 1975). However, the results of the present study do indicate that members of other classes of algae were dominant during some of the algal pulses, and therefore also involved in the declines. Seenayya (1971) reported from India that dense populations of Chlorophyta and Euglenophyta occurred in polluted ponds while diatoms and desmids were dominant in less polluted ponds. This dea is in agreement with the present study as diatoms and desmids were present in the ponds, but were never dominant.

The algal species presented in this study agree with iden-

tifications done by other workers. De Noyelles (1967) stated that phytoplankton reported from the literature as most common in small bodies of water receiving domestic sewage include species of *Chlamydomonas*, *Chlorella*, *Euglena*, *Scenedesmus*, *Ankistrodesmus* and a few blue-green algae. It would appear that the Chlorophyta and Euglenophyta are more successful at the high nutrient levels common in sewage ponds.

Dogadina (1971) reported that Cryptomonas spp. (C. ovata is mentioned) are not a major factor in the algal flora of sewage ponds, although individual species live there and withstand substantial variations in the chemical composition of the water.

From the algal composition results for system A (see Table 3) the following seasonal succession of dominant algal species appears to be evident for the twelve month study period: Chlorella sp. and Anabaena sp. were dominant during midwinter (May to July), but as temperatures increased in spring (August and early September) these species were replaced by Chlamydomonas globosa which in turn was followed by Micractinium pusillum in early summer (late September and October). Euglena sanguinea and Cryptomonas ovata together with Chlamydomonas globosa were dominant during mid-summer (November to January), and these three species exhibited fluctuating dominance during late summer and autumn (February to April) with C. globosa being individually dominant during the algal pulses observed during this period. In systems B and C no seasonal succession was apparent (Table 3) but the observations made for these two systems were only superficial and were probably not detailed enough to exhibit any seasonal trends. However, even from the superficial results it is evident that if any seasonal succession did occur in systems B and C, these successions would most certainly have been different to the succession pattern observed in system A. This is important as it indicates that the three systems are different as far as their seasonal succession of algal species composition is concerned, and this must then indicate that the physical agitation of the primary pond water (recirculation and aeration), influenced the algal composition of the pond systems. It might be this difference in the algal composition of the systems which was a major factor in causing the three systems to function in different ways, such as the differences in the chlorophyll a concentration patterns. Patil et al (1975) stated that the predominance of various species of micro-organisms is dependent on the loading factors and the physical design of sewage ponds. Moreover, the degree of pretreatment and mixing influence the speciation in the ponds.

In all the systems, one or at the most two species of algae were outstandingly dominant at times of algal pulses (Table 3). This phenomenon had been observed by another worker (Sless, 1974) who stated that in sewage ponds one algal species was usually dominant during bloom periods to such an extent that, for all practical purposes, the ponds could be considered as being monoalgal cultures.

The results also indicate, particularly in system A (see Fig. 3) that the algal species which were dominant at the peak of the pulse, were still present but seldom dominant, at the end of the pulse period. Thus it appeared that the dominant species population was reduced to a greater extent during the decline phase of the pulse periods than the other algal species that were not dominant during the incline phase of the pulses. This may indicate that, if a toxin was produced by the dominant algae during a pulse, the toxin was probably specific for the dominant species, and had little or no effect on the other species present. This phenomenon was apparently particularly true for the pulses involving *Chlamydomonas globosa* (pulses 1, 2, 7 and 8)

as the dominant a gal species in system A. No reference to the above suggestion could be found in the existing literature although *Chlamycomonas reinhardtii* is known to produce a non-specific inhibitor (Proctor, 1957).

The pulses involving Micractinium pusillum (pulses 3 and 4) did not indicate an enhanced reduction in the cell number of this species after the peak of the pulse. The decline phases of these pulses appeared to involve uniform reductions in the entire algal populations prespective of the species present. Hence it could be suggested that, if M. pusillum produces a toxic substance at peak pulse conditions, the toxin is probably non-specific.

The pulses involving . Euglena sanguinea, Cryptomonas ovata and Anabarna sp. (pulses 5, 6, 9 and 10) were all associated with a co-dominant green algal species, either Chlamydomonas grobosa or Chlorella sp. The results for these pulses are obscure, but it does seem as if the dominant algal species during these pulse periods were no longer dominant by the end of the pulses. Thus it appears as if one or more of the above algal species could have been producing autotoxins or heterotoxins. The literature contains a fair amount of evidence of toxins being produced by Euglena spp. and in particular Chlorella spp. and Anabeana spp. (Schwimmer & Schwimmer, 1964; Harris and Caldwel, 1974). However, the specificities of these toxins were not discussed.

It must be emphasized that experiments were not performed during this study to detect the presence of toxic substances produced by maturation pond algae. If toxins were not produced by the algae, then some other inhibiting factor must have been present in the ponds which inhibited the growth of the dominant algal species specifically during the decline phase of some of the note-1 algal pulses.

In a previous paper Shillinglaw and Pieterse (1977) mentioned that ammonia toxicity at pulse conditions might have caused the algal population declines. If this suggestion was correct, then the fact that the concentration of Chlamydomonas globosa was reduced to non-dominant levels during algal pulse decline periods, could indicate that C. globosa is very sensitive to high ammonia concentrations. Micractinium pusillum might not be as sensitive to ammonia toxicity, hence the concentration of this species was rot reduced to non-dominant levels during algal pulse declines. Furthermore, if ammonia toxicity is inhibiting algal growth in maturation ponds, aeration may have removed ammonia from system C resulting in higher chlorophyll a concentrations.

One interesting feature from the results (Table 3) is that colonial algal species appeared more frequently as the dominant algae in systems B ard C than in system A where the unicellular algae were most frequently dominant. Although no definite explanation can be offered for this phenomenon, it can be suggested that the agitation of the primary pond water directly favoured the colonial algal species, possibly facilitating the suspension of the large colonies. Since Shillinglaw and Pieterse (1977) illustrated that zooplankton could influence the algal concentration, it is also possible that the agitated conditions in systems B and C resulted in differences in the composition and concentration of zooplankton populations which could differentially influence the composition of the algal populations in the agitated and non-agitated systems.

# Conclusions

1. The algal populations of all three systems exhibited wax

and wane patterns.

- 2. The algal concentration declines occurred during both summer and winter months of the study period.
- 3. The aerated system exhibited the highest mean chlorophyll a concentration for the study period. This system also had the highest algal concentration index which suggests that the conditions in this system were most favourable for algal growth.
- 4. Aeration appears to reduce the number of algal concentration pulses in the system. In the aerated system the algal concentration remained more constant and seldom declined to low concentrations.
- 5. Recirculation of the primary pond water did not increase the algal concentration.
- 6. Members of the *Chlorophyceae* were the most dominant types of algal species in the pond systems.
- Members of the Chlorophyceae, Euglenophyceae, Cryptophyceae and Cyanophyceae were dominant during algal pulses and thus during the ensueing algal concentration decline periods.

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#### References

- DE NOYELLES, Jr. F. (1967) Factors affecting phytoplankton distribution in a double-cell sewage lagoon. J. Phycol. 31 174-181.
- DOGADINA, T.V. (1971) Pyrrophytic algae in sewage. Kharkov University Press. Kharkov, USSR.
- HARRIS, D.O. and CALDWELL, H.D. (1974) A study of naturally occurring algicides produced by freshwater algae. University of Kentucky Water Resources Research Institute, Lexington, Kentucky. Report No. 79. 31 pp.
- KEIRN, M.A. (1974) Application of algal and plant systems for the treatment of nutrient enriched waters. Unpublished project report. July 1973 February 1974. Project No. 414. NIWR, CSIR.
- KEIRN, M.A. (1975) Application of algal and plant systems for the treatment of nutrient enriched waters. Unpublished project report. February 1974 — January 1975. Project No W6/153/3. NIWR, CSIR.
- MARKER, A.F.H. (1972) The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. Freshwater Biol. 2 361-385.
- PATIL, H.S., DODAKUNDI, G.B. and RODGI, S.S. (1975) Succession in zoo- and phytoplankton in a sewage stabilization pond. Hydrobiologia 47 253-264.
- PENNAK, R.W. (1946) The dynamics of freshwater plankton populations. Ecol. Monogr. 16 339-355.
- PROCTOR, V.W. (1957) Studies of algal antibiosis using Haemato-coccus and Chlamydomonas. Limnol. Oceanogr. 2 125-139.
- SCHWIMMER, D. and SCHWIMMER, M. (1964) Algae and medicine. In: Jackson, D.F. Ed. Algae and man. Plenum Press, New York.
- SEENAYYA, G. (1971) Ecological studies in the plankton of certain freshwater ponds of Hyderabad India II, Phytoplankton 1. Hydrobiologia 37 55—88.
- SHILLINGLAW, S.N. and PIETERSE, A.J.H. (1977) Observations on algal populations in an experimental maturation pond system. Water S.A. 3(4) 183-192.
- SLESS, J.B. (1974) Biological and chemical aspects of stabilization pond design. Rev. Environ. Health 1 327-354.
- UTERMÖHL, H. (1958) Zur Vervollkommung der quantitativen Phytoplankton Methodik. Mitt. Int. Verein. Limnol. 9 1-38.
- VAN DER POST, D.C. and TOERIEN, D.F. (1974) The retardment of algal growth in maturation ponds. Wat. Res. 8 593-600.
- VOLLENWEIDER, R.A. (1969) A manual on methods for measuring primary production in aquatic environments. IBP handbook No 12. Blackwell Scientific Publications, Oxford-Edinburgh. 213 pp.