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# Phosphorus and Nitrate Removal by Selected Wastewater Protozoa Isolates

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**Abstract:** In the present study, the efficiency of ten selected wastewater protozoa in the removal of phosphorus and nitrogen and their effects on other physico-chemical parameters was investigated. Protozoa were isolated from the aerobic zone of Daasport wastewater treatment plant, in Pretoria, South Africa, using the modified Chalkey's medium. The isolates were screened for nutrient removal ability in shake flasks, at laboratory temperatures for 96 h. During each sampling, aliquot samples were collected for the analysis of phosphorus, nitrate-nitrogen, Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), pH, of the mixed liquor including the growth rate of the isolates using standard methods. The results indicated a steady increase in growth rate of the isolates and all the isolates used in this study showed some measure of phosphorus and nitrate removal ability. In the presence of the test protozoa isolates, an increase in COD and a decrease in DO were noted in the mixed liquor inoculated with the organisms, while the pH only increases slightly. This study revealed a significant correlation between growth rate and nutrient removal ($R = -0.806$, $p<0.001$ for phosphorus; $R = -0.799$, $p<0.01$ for nitrate). The present study has been able to show that certain protozoa isolates have the ability to biologically remove phosphorus and nitrate from effluent. Consequently, it would be necessary to identify what strains of protozoa are capable of excess phosphorus and nitrogen uptake for the treatment of wastewater.

**Key words:** Phosphorus, nitrate, removal, protozoa, wastewater

## INTRODUCTION

One major drawback of an activated sludge production is the presence of high concentrations of nitrogen and phosphorus, thus making it difficult to process the sludge (Ratsak *et al*., 1996). Since the assurance of water quality has become an integral part of environmental quality management today, wastewater treatment is, therefore, one of the strategies for water quality management. Many wastewater treatment plants have been upgraded and designed to remove nutrients by the addition of chemicals (Abraham *et al*., 1997; Spellman, 1997; Bitton, 1999). However, chemical precipitation increases the volume of sludge produced and often results in sludge with a poor settling and dewatering characteristics (Petersen *et al*., 1998; Amir, 2004).

Biological nitrogen and phosphorus removal from wastewater is an essential treatment to avoid unpleasant conditions for natural resources (Pitman, 1982; Horan, 1990; Vander Post and Schutte, 2003). Enhanced biological phosphorus removal has been considered to depend on polyphosphate bacteria that are able to accumulate polyphosphate by storing more phosphorus than they need for growth (Lydia, 2006). Although nitrifying bacteria are known to oxidize ammonia-nitrogen to nitrate-nitrogen in a two-stage conversion process, very little energy is derived from these oxidation reactions. In fact, energy required to converting $CO_2$ to cellular carbon and nitrifying bacteria represent a small percentage of the total population of microorganisms in activated sludge (Choubert *et al*., 2005).

Protozoa play important roles in the removal of pathogens from wastewaters, as well as being indispensable in the reduction of chemical oxygen demand of wastewaters, resulting in good quality effluent (Curds and Cockburn, 1970). They are also known to be important consumers of bacteria in wastewater purification systems, such as activated sludge plants (Fried and Lemmer, 2003). They are useful biological indicators of the condition of the activated sludge. Being strict aerobes, they prove to be excellent indicators of an aerobic and toxic environment, as they exhibit a greater sensitivity to toxicity than to bacteria (Madoni *et al*., 1996).

There is paucity of information on the roles protozoa play in the eventual removal of phosphorus and nitrate

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from wastewater systems. Knowledge of these roles will help in an effective biological wastewater treatment, since the reduction of phosphorus and nitrogen to the lowest possible level is known to be vital to the maintenance of unpolluted water supplies. Hence the aim of this study was to investigate the phosphorus and nitrate removal efficiency of some selected wastewater protozoa isolates. The effect of the isolates on some of the water quality parameters (such as chemical oxygen demand, dissolved oxygen and pH) was also investigated.

## MATERIALS AND METHODS

For isolation of protozoa, mixed liquor sample was collected from the aerobic zone of Daasport wastewater treatment plant in Pretoria, South Africa between November and December 2006. The mixed liquor was sonicated for 3 min to release protozoa that were trapped between flocs. A 250 mL sub-sample of the mixed liquor was then condensed to 10 mL over an 8 mm pore-size, membrane filter (SC, 45 mm diameter, Millipore Corp). Live samples were concentrated by using the same factor (25:1) in a series of 30 mL sub-samples, with a swinging bucket refrigerated rotor (1200 rpm for 5 min). The protozoa were then isolated using a stereo microscope (Axiovert, S100, Carl Zeiss GmbH) under 40 X objective by picking them with a hand glass capillary and visualizing them using a light microscope.

To remove all eukaryotes and detritus, protozoa isolates were washed 3-5 times in sterile filtered mixed liquor sample from the aerobic zone, placed inside the surface of a sterile Petri dish. The isolated protozoa were then transferred to microtitre plates with flat bottom, containing Chalkley's medium (UNKCC, 2001). Axenic cultures were obtained by repeated passages in Chalkley's medium that was supplemented with penicillin (66 μg $mL^{-1}$) and heat-killed *E. coli*. WG4. The isolates were incubated at 25±2°C in the dark. The experimental setup was done in a laminar flow under aseptic conditions.

Culture medium for nutrient uptake studies was mixed liquor sample from the anaerobic zone of Daasport wastewater treatment plant in Pretoria. For nutrient uptake studies, the mixed liquor was allowed to settle for 2 h, after which it was filtered, using Whatman No. 1 filter papers, to remove biomass and other suspended solids. The following salts, sodium acetate (5 g $L^{-1}$), $MgSO_4$. $7H_2O$ (0.5 g $L^{-1}$) and $KNO_3$ (0.18 g $L^{-1}$) were added to the filtrate (Bosch and Cloete, 1993; Momba and Cloete, 1996). The medium was prepared in ten 1 L flasks and then autoclaved, after which it was dispensed into 250 mL Erlenmeyer flasks in a laminar flow. Prior use, the Erlenmeyer flasks were autoclaved, after which they were dried in a hot-air oven for 1 h at 150°C.

Isolates were screened for nutrient uptake by inoculating each flask containing the mixed liquor with them. Prior inoculation, the sterilized medium was previously cultured on nutrient agar plates and incubated for 24 h at 37°C. This was done to ascertain the effectiveness of the sterilization process and to be sure no microbial contaminant was present. Flasks with plates without any growth were inoculated with the test isolates. The inoculated flasks were incubated in a rotary shaker (model P-Selecta) and agitated at a shaking speed of 100 rpm at 25°C. Aliquot samples (20 mL) were collected from each flask every 3 h for the first 12 h and thereafter every 24 h for 96 h, for the determination and estimation of phosphorus, nitrate-nitrogen, Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), pH and growth rate, using standard methods (APHA, 2001).

Chemical Oxygen Demand (COD) was measured using the closed reflux colorimetric method, as described in standard methods (APHA, 2001). To a Teflon-coated tube, 2.5 mL of the sampled mixed liquor was added, after which 1.5 mL of digestion solution (10.2 g $L^{-1}$ $K_2Cr_2O_7$, 170 mL $L^{-1}$ concentrated $H_2SO_4$ and 33.3 g $L^{-1}$ $HgSO_4$) and 3.5 mL of concentrated $H_2SO_4$ were added. The tubes were placed in a COD reactor (HACH COD reactor) and refluxed for 2 h at 150°C. The tubes were allowed to cool and absorbance was read using a spectrophotometer (DR Lange Spectrophotometer, model CADAS 50 S) at a wavelength of 600 nm. The absorbances of the samples were read along with potassium hydrogen phthalate standards that ranged from 0 to 1000 mg COD $L^{-1}$. The following formula was used to calculate the COD level of samples

$$\text{COD (mg L}^{-1}) = \frac{\text{mg in final volume} \times 1000}{\text{Sample volume}}$$

Nitrate-nitrogen was measured using the salicylate method. To a 100 mL glass beaker, 5 mL aliquot of the sample was added, followed by 2 mL of 0.5 % sodium salicylate and then the beakers were placed in a water bath heater; to evaporate to dryness. After evaporation, they were cooled and 1 mL of concentrated $H_2SO_4$ was added, while tilting the beakers to wet the bottom and lower edges with the $H_2SO_4$ completely, thereafter they were allowed to stand for 10 min before adding 10 mL of NaOH (50% w/v) solution. Each solution in a beaker was

transferred to a separate 100 mL volumetric flask by rinsing the beakers a couple of times with distilled $H_2O$. Absorbance of the samples, together with those of $KNO_3$ standards were read at a wavelength of 410 nm, using a spectrophotometer (DR Lange Spectrophotometer, model CADAS 50 S). Nitrate-nitrogen was later calculated as:

$$mg\text{-}NO_3\ L^{-1} = \text{Concentration from calibration curve} \times D$$

Where:
D = Dilution factor.

The ascorbic acid method was used for the estimation of phosphorus. To a 50 mL portion of the diluted sample in a 100 mL volumetric flask, 13 mL of combined reagent (for 100 mL of combined reagent: 50 mL 5 N $H_2SO_4$, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution and 30 mL 0.001 M ascorbic acid solution) was added. The flasks were allowed to stand for 30 min to allow for colour development, after which the absorbance values were read at a wavelength of 880 nm. Potassium dihydrogen phosphate standards were read together with the samples. Phosphorus was estimated as:

$$mg\text{-}PO_4\ L^{-1} = \text{Concentration from calibration curve} \times D$$

Where:
D = Dilution factor.

The pH and DO of the samples were measured using an electric pH meter with glass electrode (ORION, model 410A) and dissolved oxygen metre (HACH model Sension 8), respectively.

Growth rate of the isolates during incubation was calculated, using the following formula as described by Coran and Dola (1994).

$$\text{Growth rate } (day^{-1}) = \frac{(InC_1 - InC_0}{(t_1 - t_0)}$$

Where:
$C_0$ and $C_1$ = The concentrations of protozoa in the beginning and at the end of incubation period.
$t_1$-$t_0$ = Represents elapsed time between selected intervals.

Data were analyzed statistically using the SPPS computer software, version 11.0. Test of relationship was carried out using the Pearson correlation index at 95 and 99% confidence interval.

## RESULTS

Table 1 shows, a steady increase in growth rate of the isolates was observed throughout the period of study. This trend was noted in all isolates. The highest growth rate of 4.32 $d^{-1}$ was observed in isolate C and G, while lowest growth rate of 3.09 $day^{-1}$ was observed in isolate I.

All the isolates used in this study showed the ability to remove phosphorus at the end of the 96 h incubation period, when compared with the control (Table 2). At the expiration of incubation, isolate H showed a phosphorus removal efficiency of 94.08% (decreasing from an initial 79.40 to 4.70 mg $L^{-1}$), while the least phosphorus removal efficiency of 18.83% was shown by isolate J (decreasing from an initial 79.92 to 64.87 mg $L^{-1}$), as shown in Table 6.

In general, as observed in phosphorus, all the isolates showed a degree of nitrate removal at the end of the 96 h incubation period (Table 3). Isolate B showed the highest nitrate removal efficiency of 99.10% (decreasing from 13.39 mg $L^{-1}$ at time 0 h to 0.12 mg $L^{-1}$ after 96 h), while isolate J showed the least removal efficiency of 20.33% (decreasing from 12.10 mg $L^{-1}$ at time 0 to 9.64 mg $L^{-1}$, after 96 h), as shown in Table 6.

Table 1: Growth rate of the test protozoan isolates when inoculated in the mixed liquor samples

| | Time (h) | | | | |
|---|---|---|---|---|---|
| Isolates | 0 | 24 | 48 | 72 | 96 |
| A | 0 | 0.95 | 1.04 | 2.53 | 4.09 |
| B | 0 | 0.69 | 2.33 | 3.44 | 4.00 |
| C | 0 | 0.79 | 1.76 | 3.36 | 4.32 |
| D | 0 | 1.04 | 1.77 | 3.50 | 3.88 |
| E | 0 | 1.56 | 2.19 | 2.63 | 3.37 |
| F | 0 | 1.65 | 1.59 | 3.09 | 3.89 |
| G | 0 | 1.41 | 2.46 | 3.62 | 4.32 |
| H | 0 | 1.43 | 2.25 | 3.06 | 3.96 |
| I | 0 | 0.94 | 2.23 | 2.64 | 3.09 |
| J | 0 | 1.00 | 2.10 | 3.09 | 3.60 |

*Values are average of duplicate samples and are expressed as $d^{-1}$. A-J represents the test isolates

Table 2: Average phosphorus removal by the test isolates, before and after 96 h incubation

| | Initial phosphorus | Final phosphorus |
|---|---|---|
| Isolates | (mg $L^{-1}$) | |
| A | 74.46 (±0.34) | 48.52 (±3.31) |
| B | 76.56 (±0.10) | 7.16 (±0.28) |
| C | 76.46 (±0.05) | 12.20 (±0.50) |
| D | 73.24 (±0.09) | 41.89 (±2.21) |
| E | 79.06 (±0.25) | 41.38 (±1.55) |
| F | 76.42 (±0.49) | 9.60 (±0.93) |
| G | 79.40 (±0.25) | 7.73 (±0.83) |
| H | 79.40 (±0.25) | 4.70 (±0.79) |
| I | 76.77 (±0.49) | 55.32 (±1.33) |
| J | 79.22 (±0.23) | 64.87 (±0.83) |
| Control | 72.21 (±0.53) | 70.80 (±0.79) |

*: Values in parenthesis are ±standard deviation. All values are average of duplicate samples. Initial concentration was at time 0 while final concentration was after 96 h. A-J represents protozoan isolates while control is uninoculated

Table 3: Average nitrate removal by the test isolates, before and after 96 h incubation

| Isolates | Initial phosphorus (mg $L^{-1}$) | Final phosphorus (mg $L^{-1}$) |
|---|---|---|
| A | 13.77 (±0.04) | 6.25 (±0.16) |
| B | 13.39 (±0.02) | 0.12 (±0.02) |
| C | 13.12 (±0.07) | 0.29 (±0.07) |
| D | 13.70 (±0.04) | 8.57 (±0.17) |
| E | 12.73 (±0.04) | 3.13 (±0.04) |
| F | 12.49 (±0.04) | 1.23 (±0.02) |
| G | 12.30 (±0.01) | 3.10 (±0.01) |
| H | 12.30 (±0.01) | 2.00 (±0.02) |
| I | 12.53 (±0.01) | 6.37 (±0.13) |
| J | 12.10 (±0.01) | 9.64 (±0.06) |
| Control | 13.65 (±0.02) | 13.57 (±0.03) |

*: Values in parenthesis are ± standard deviation. All values are average of duplicate samples. Initial concentration was at time 0 while final concentration was after 96 h. A-J represents protozoan isolates while control is uninoculated

Table 4: Average COD concentration in mixed liquor inoculated by the test isolates, before and after 96 h incubation

| Isolates | Initial COD (mg $L^{-1}$) | Final COD (mg $L^{-1}$) |
|---|---|---|
| A | 155.90 (±3.01) | 682.60 (±13.17) |
| B | 161.24 (±4.53) | 762.93 (±11.31) |
| C | 139.87 (±1.50) | 785.22 (±10.01) |
| D | 153.76 (±6.03) | 567.24 (±4.17) |
| E | 139.55 (±6.04) | 626.28 (±11.12) |
| F | 161.61 (±4.53) | 535.28 (±9.69) |
| G | 187.23 (±1.51) | 632.81 (±11.91) |
| H | 194.65 (±9.14) | 712.70 (±14.93) |
| I | 145.96 (±6.03) | 773.77 (±11.84) |
| J | 194.71 (±9.06) | 783.85 (±10.52) |
| Control | 113.93 (±3.02) | 70.80 (±4.14) |

*: Values in parenthesis are ± standard deviation. All values are average of duplicate samples. Initial concentration was at time 0 while final concentration was after 96 h. A-J represents protozoan isolates while control is uninoculated

Table 5: Average DO concentration in mixed liquor inoculated by the test isolates, before and after 96 h incubation

| Isolates | Initial DO (mg $L^{-1}$) | Final DO (mg $L^{-1}$) |
|---|---|---|
| A | 4.03 (±0.01) | 0.08 (±0.14) |
| B | 4.18 (±0.00) | 0.05 (±0.11) |
| C | 4.18 (±0.01) | 0.04 (±0.13) |
| D | 4.18 (±0.01) | 0.06 (±0.17) |
| E | 4.00 (±0.01) | 0.05 (±0.14) |
| F | 3.81 (±0.07) | 0.07 (±0.17) |
| G | 3.70 (±0.03) | 0.05 (±0.15) |
| H | 3.61 (±0.03) | 0.06 (±0.12) |
| I | 3.50 (±0.03) | 0.09 (±0.18) |
| J | 3.62 (±0.08) | 0.07 (±0.10) |
| Control | 4.15 (±0.05) | 3.18 (±0.09) |

*: Values in parenthesis are ± standard deviation. All values are average of duplicate samples. Initial concentration was at time 0 while final concentration was after 96 h. A-J represents protozoan isolates while control is uninoculated

In the presence of the test protozoa isolates, an increase in COD was noted in the mixed liquor. This was irrespective of the isolates. As can be seen from Table 6, the highest and the least COD increase of 461.39 and 231.22% were recorded for isolates C and F, respectively. These percentages translate COD concentrations

Table 6: Percentage $PO_4$, $NO_3$, DO and COD concentrations removed from mixed liquor by the different isolates

| Isolates | $PO_4$ | $NO_3$ | DO | COD |
|---|---|---|---|---|
| A | 34.84 | 54.61 | 98.01 | 337.34 |
| B | 90.65 | 99.10 | 98.80 | 373.16 |
| C | 84.04 | 97.79 | 99.04 | 461.39 |
| D | 42.80 | 37.45 | 98.56 | 268.91 |
| E | 47.66 | 75.41 | 98.75 | 348.79 |
| F | 87.44 | 90.15 | 97.80 | 231.22 |
| G | 90.26 | 74.80 | 98.65 | 237.99 |
| H | 94.08 | 83.74 | 98.34 | 266.14 |
| I | 27.94 | 49.16 | 97.43 | 430.12 |
| J | 18.83 | 20.33 | 98.07 | 302.57 |
| Control | 1.95 | 0.59 | 23.37 | 7.27 |

*: Values are in percentages and are average of duplicate samples. Apart from COD that reflects % increase, all others are % decreases at the end of incubation

Table 7: pH variations among mixed liquor with protozoa isolates

| Isolates | Time (h) 0 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|
| A | 7.5 | 7.8 | 8.0 | 8.1 | 8.0 |
| B | 7.6 | 7.7 | 8.0 | 8.0 | 8.0 |
| C | 7.6 | 7.7 | 8.0 | 8.0 | 8.0 |
| D | 7.7 | 7.7 | 8.0 | 8.1 | 8.4 |
| E | 7.8 | 7.8 | 8.4 | 8.5 | 8.5 |
| F | 7.8 | 7.8 | 8.5 | 8.5 | 8.4 |
| G | 7.8 | 7.8 | 8.3 | 8.4 | 8.4 |
| H | 7.7 | 7.8 | 8.3 | 8.4 | 8.4 |
| I | 7.8 | 7.8 | 8.3 | 8.4 | 8.4 |
| J | 7.7 | 7.9 | 8.2 | 8.2 | 8.2 |

*: Values are average of duplicate

increasing from 161.61 to 535.28 mg $L^{-1}$ and from 139.87 to 785.22 mg $L^{-1}$ in mixed liquor containing isolate F and C, respectively, at the end of 96 h incubation (Table 4).

As shown in Table 5 and 6, DO values decreased drastically in the mixed liquors containing the isolates. This decrease in DO was irrespective of the test isolates. All the mixed liquor inoculated with the test isolates showed a decrease of DO concentration that was greater than 95%, as against the control that had only a decrease level of 23.77%.

Table 7 shows the variation in pH during the period of incubation. The pH values of the mixed liquor inoculated with the test isolates only increased slightly. From an initial pH of 7.6 in mixed liquor containing isolate C, the pH only increased to 8.0 at the end of the incubation period. A similar trend was observed in all the isolates.

## DISCUSSION

The protozoa isolates that were used in this study were indigenous species. The use of indigenous microbial communities have been known to increase the likelihood that important community and ecosystem level processes will be functioning in the test system (Cairns and McCormick, 1991).

The present study has shown that protozoa have the ability of removing phosphorus and nitrate in mixed liquor. Earlier workers have linked protozoa to enhanced nutrient mineralization in aquatic microcosms (Barsdate *et al*., 1974; Ratsak *et al*., 1996). In a study carried out by Johannes (1965) on the influence of marine protozoa on nutrient regeneration, protozoa were reported to be responsible for phosphorus excretion and uptake. In the same study, members of the micro and nano-zooplanktons, (ciliates and flagellates) were suggested to be probably very important in nutrient excretion, specifically phosphorus dynamics in marine systems. Dola (1997) have reported that nutrients utilized in the formation of biomass have either been recycled (i.e., are derived from consumption and excretion by consumers of particulate matter, primarily protozoa) or have been delivered to the surface. Although nutrient removal has been mainly linked to bacteria, it has been reported that protozoa in activated sludge live by the direct removal of organic nutrients when bacteria are unavailable (Johannes, 1965; Curds and Cockburn, 1970; Hiroki *et al*., 1995). In this study, the test protozoa that were inoculated in the mixed liquor were not fed with either live or heat-killed bacteria, therefore the chances of bacteria contaminant and involvement in nutrient removal in the mixed liquor was eliminated.

The protozoa growth rate in this study increased throughout the incubation period. Sidat *el al.* (1999) has reported that during biological wastewater treatment, the active biomasses are responsible for nutrient removal. In the present study, the active biomasses were the test protozoa isolates. This study revealed a significant correlation between growth rate and nutrient removal ($R = -0.806$, $p<0.001$ for phosphorus; $R = -0.799$, $p<0.01$ for nitrate). A similar trend has been reported by earlier workers (Petropoulous and Gilbride, 2005). Other workers have shown that increasing biomass concentration (i.e., bacterial biomass) increases nutrient uptake capacity, due top an increase utilization rate of the organisms (Momba and Cloete, 1996; Sidat *et al*., 1999).

Although the effectiveness of protozoa in wastewater purifying processes has been reported to be due to the fact that they feed on bacteria, the presence of protozoa (ciliates and flagellates) have been found to serve as useful indicators of parameters, other than effluent quality in wastewater purifying process, such as denitrification (Salvado *et al*., 1995; Johanna *et al*., 1999). The association of bacteria and protozoa has been reported to play significant role in nutrient recycling in marine environments (Strauss and Dodds, 1997). There is also increasing evidence that primary production in oceans relies mostly on nutrients recycled in the euphotic zone by protozoa, rather than by bacteria (Glibert, 1982; Coran and Dola, 1994).

Nitrogen regeneration by protozoa in aquatic environment has been reported (Keith *et al*., 2005). Caron and Dola (1994) have reported then involvement of flagellates in nitrogen assimilation or remineralization. Although protozoa role in nitrification have mostly been linked to their grazing on bacterial population, Petropoulos and Gilbride (2005) have reported that the roles protozoa play in natural ecosystems is highly underestimated. The most significant nitrogen mechanisms from wastewaters are considered to be nitrification and denitrification (Strang and Wareham, 2005). In this study, only denitrification was monitored.

Despite the fact that some workers (Lee and Welander, 1996) have reported a decrease in COD concentration in their studies, none of the isolates used in this study showed COD removal efficiency in the mixed liquor. This disparity may have due to the type of wastewater used or on the initial COD concentration in their study. In a study conducted by Kasia *et al*. (2005), COD removal efficiency was low when biological removal of nitrogen species from metal processing wastewater was investigated.

This study revealed a significant negative correlation between COD and nutrient removal ($R = -0.547$, $p<0.01$ for phosphorus; $R = -0.668$, $p<0.01$ for nitrate). However, COD concentration was positively correlated with growth rate of the test isolates ($R = 0.806$, $p<0.01$). According to Pitman (1982), sewage characteristic is reported to have a significant influence on biological nutrient removal. If the COD concentration is high, excess COD becomes available to allow for complete denitrification, but when COD is low, denitrification will not be complete. In the present study, COD values were high. Sponza and Atalay (2005) have reported in their study that as COD values increased, there was a decrease in phosphorus concentration. A similar trend was observed in this study.

There was a negative significant correlation ($R = -0.602$, $p<0.01$) between COD and DO in this study. DO and growth rate were also observed to be negatively significant ($R = -0.825$, $p<0.01$). The decrease in DO values in the presence of active biomass have been reported by other workers (Curds and Cockburn, 1970; Petropolous and Gilbride, 2005).

The slight increase in pH of the mixed liquor containing the test isolates, as observed in this study, have been reported by Chevalier *et al*. (2000). In their report, pH increase substantially in all of their growing cultures. pH values in this study were within neutral and

low alkaline ranges. It has been reported that high pH values are likely to induce phosphorus precipitation and that phosphorus removal is rapid in culture media at high pH values (Diamadopoulos and Benedek, 1984; Laliberte *et al*., 1998; Chevalier *et al*., 2000).

## CONCLUSIONS

This preliminary study has been able to show that, certain wastewater protozoa isolates have the ability to remove phosphorus and nitrate from wastewater in a shake flask environment, which was previously not well known. The study has still been able to show the effect of protozoa isolates on other physico-chemical properties of wastewater. Because the study was carried out under laboratory temperatures, the effect of temperature on removal of the nutrients by the isolates was not investigated.

Detailed studies are currently in progress on the isolates to ascertain the optimum pH and temperature conditions for nutrient uptake by the protozoan isolates, both in shake flasks and microcosms as well as to identify the protozoan species that can be used for enhanced phosphorus and nitrate removal.

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