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Relationship of Carbon Source Concentration to Nutrient Uptake by Protozoa in Activated Sludge Mixed Liquor

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Abstract: The present study was aimed at investigating the relationship between carbon source concentration and nutrient uptake efficiency of three protozoan isolates. Three carbon sources (acetate, glucose and sucrose) were used at respective concentrations of 5, 10 and 15 g L^{-1} for this investigation. The study was carried out in a shake flask at a temperature of 25°C. Aliquot samples were collected at time zero and every 24 h for the estimation of phosphate, nitrate, Chemical Oxygen Demand (COD), Dissolved Oxygen (DO) and growth rate. Results revealed a significant phosphate and nitrate uptake at carbon source concentration of 5 g L^{-1}. This was irrespective of isolates or carbon source used. The results also showed that with a high initial carbon source concentration, initial COD was observed to be high. In the presence of acetate, COD removal from mixed liquor was not observed. However in the presence of glucose or sucrose, COD decrease was observed. There was no observed significant variation ($p \leq 0.05$) between DO values at the different concentrations of carbon source. A similar trend was also observed for growth rate. This study had been able to give an insight into the optimum carbon source concentration in mixed liquor that will enhance nutrient uptake by the test protozoa.

Key words: Carbon source, nutrient uptake, phosphate, nitrate, COD

INTRODUCTION

An integral part of environmental quality management in the world today is the assurance of water quality, of which wastewater treatment is one of the strategies (Mbewele, 2006). One of the important factors affecting water quality is the enrichment of nutrients in water bodies. The discharge of wastewater with a high nitrogen and phosphorus level often result in eutrophication of receiving water bodies, such as lakes and rivers (Danaleurich *et al*., 1998; Mulkerrins *et al*., 2004).

Nutrient removal in the past has been majorly through the use of chemicals, which is expensive. Presently, biological nutrient removal processes have been widely used for the treatment of wastewater with high concentrations of nitrogen, phosphorus and chemical oxygen demand and this have been found to be economically feasible (Ohkate *et al*., 1985; You and Ouyang, 2005; Yong-Zhan *et al*., 2006).

Wastewater composition has been known to be one of the most important factors affecting the performance of biological nutrient removal systems (Warangkana and Randall, 1997). Successful biological nutrient removal depends on the stability of influent wastewater flow and with sudden drastic changes to the system being avoided (Mulkerrins *et al*., 2004). Carbon sources are required for energy generation in nutrient removal systems and reports have shown that a combination of several carbon sources may result in improved performance of a sequencing batch reactor operation for nutrient removal (Kargi and Uygur, 2003).

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Several studies have shown the importance of acetate, glucose and other carbon sources in nutrient removal systems (Carucci *et al*., 1997; Tasli *et al*., 1997; Kargi and Uygur, 2003). However, not much has been reported on the relationship between carbon source concentrations to nutrient uptake by protozoa.

This study was therefore aimed at ascertaining the relationship of concentrations of sodium acetate, glucose or sucrose to nutrient uptake efficiency of three wastewater protozoa.

MATERIALS AND METHODS

Three protozoan isolates, which were obtained from Daasport wastewater treatment plant in Pretoria, South Africa, were used as the test isolates in this study. The isolates have previously been screened for phosphate and nitrate removal efficiency (Akpor *et al*., 2007) and were made up of two ciliates and one flagellate. They were presumptively identified as *Aspidisca* sp. (A) *Chilophyllum* sp. (B) and *Paranema* sp. (C).

Wastewater used for this investigation was mixed liquor from the anaerobic zone of the same treatment plant, collected between August and November 2007. Prior use, the mixed liquor was filtered (using Whatman No. 1 filter paper) and supplemented with a known concentration of a carbon source and KNO_3 (0.18 g L^{-1}) and $MgSO_4$. $7H_2O$ (0.5 g L^{-1}). Three carbon sources (sodium acetate, glucose and sucrose) were used in this study at concentrations of 5, 10 and 15 g L^{-1}, respectively.

After addition of the carbon source and the other salts, the mixed liquor was adjusted to a pH of 8.0 (±1) and then dispensed in 200 mL quantity into 250 mL capacity Erlenmeyer flasks and autoclaved. The following antibiotics: penicillin, tetracycline and streptomycin were added to each flask in concentrations of 10, 100 and 66 μg mL^{-1}, respectively. This was done to prevent any development of bacteria during the experiment. All flasks were incubated in a rotary shaker at a shaking speed of 100 rpm at incubating temperature of 25°C.

After inoculation with test isolates, aliquot samples of mixed liquor were withdrawn aseptically from each flask at time 0 h and every 24 h, for the next 96 h, for the estimation of total phosphate, nitrate-nitrogen, Chemical Oxygen Demand (COD), Dissolve Oxygen (DO) and growth rate, using standard methods (APHA, 2001).

Total phosphate, COD and nitrate-nitrogen were estimated, using the ascorbic acid, closed reflux and salicylate methods, respectively, as described in standard methods (APHA, 2001). Dissolved oxygen was determined, using a dissolved oxygen (model Session 8), while growth rate was calculated as described by Coran and Dola (1994).

All statistics analysis was carried out using the SPSS computer software 11.0. Comparison of means was done using the One-Way Analysis of Variance (ANOVA).

All experimental setups were carried out in triplicate.

RESULTS

In mixed liquor inoculated with isolate A, phosphate uptake was observed to be highest at acetate concentration of 5 g L^{-1}; decreasing from 74.12 to 11.72 mg L^{-1}, after 96 h (Fig. 1). A similar trend was observed in the presence of sucrose, decreasing from 64.86 to 10.30 mg L^{-1}, after 96 h incubation. However, in the presence of glucose as carbon source, phosphate uptake was highest at a concentration of 10 g L^{-1}, decreasing from 71.98 to 7.53 mg L^{-1} (Fig. 1).

The difference in phosphate uptake by isolate A at different concentrations of acetate was observed to be highly significant ($p \leq 0.05$). In the presence of either glucose or sucrose, although significant variation was observed between other concentrations, the difference between 5 g L^{-1} and 10 g L^{-1} were not observed to be significant ($p \leq 0.05$).

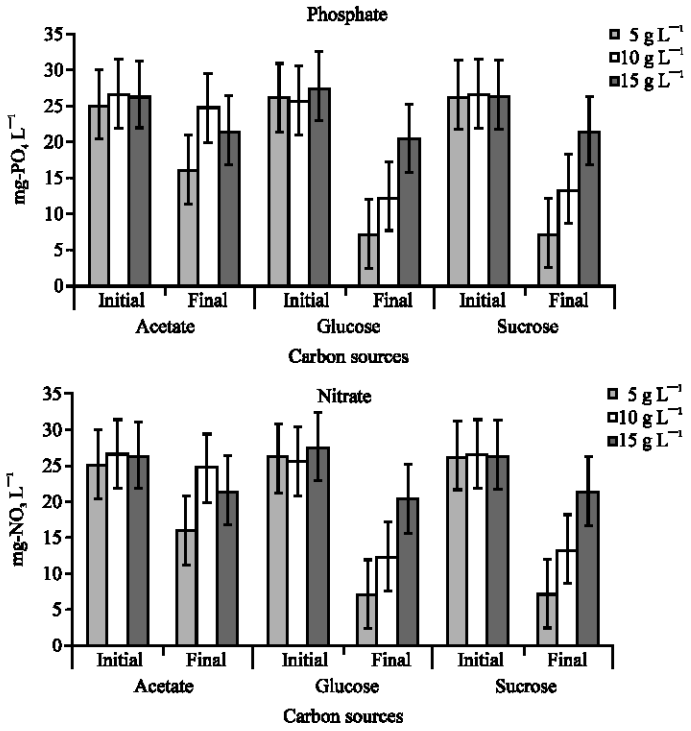

Fig. 1: Phosphate and nitrate uptake by isolate A at different concentrations of the carbon sources (acetate, glucose and sucrose) in mixed liquor. Initial and final means concentration at time zero and after 96 h incubation, respectively

As observed in phosphate uptake, nitrate uptake was observed to be highest at concentration of 5 g L^{-1}. This was irrespective of the carbon sources, thus decreasing from 25.02 to 15.95 mg L^{-1}, 26.12 to 7.04 mg L^{-1} and 26.09 to 7.04 mg L^{-1}; in the presence of acetate, glucose and sucrose, respectively (Fig. 1).

There was a highly significant difference between nitrate uptakes at the different concentrations of acetate ($p \leq 0.05$). However, in the presence of glucose or sucrose, significant differences were not observed between concentrations of 10 and 15 g L^{-1} (glucose) and between 5 and 10 g L^{-1} (sucrose) at the same probability level.

As shown in Fig. 2, phosphate uptake in mixed liquor containing isolate B was observed to be highest at 5 g L^{-1} of acetate, glucose or sucrose. This shows a decrease in phosphate from 73.52 to 15.47 mg L^{-1}, 63.82 to 11.19 mg L^{-1} and 71.35 to 13.83 mg L^{-1}, in the presence of acetate, sucrose and glucose, respectively.

In the presence of acetate, there was a significant variation between uptake at concentrations 5 g L^{-1} and either 10 or 15 g L^{-1} ($p \leq 0.05$). However, in the presence of glucose, significant variation was observed among the different concentrations while in the presence of sucrose, no variation was observed between concentrations of 5 and 10 g L^{-1}. As observed in phosphate, nitrate uptake was also observed to be highest at carbon source concentration of 5 g L^{-1}. This trend was common in the presence of acetate, glucose and sucrose. At the end of 96 h incubation for carbon source concentration at 5 g L^{-1} nitrate concentration decreased from 25.62 to 9.72 mg L^{-1}, 25.38 to 6.11 mg L^{-1} and 26.38 to 5.11 mg L^{-1}, for acetate, glucose and sucrose, respectively (Fig. 2).

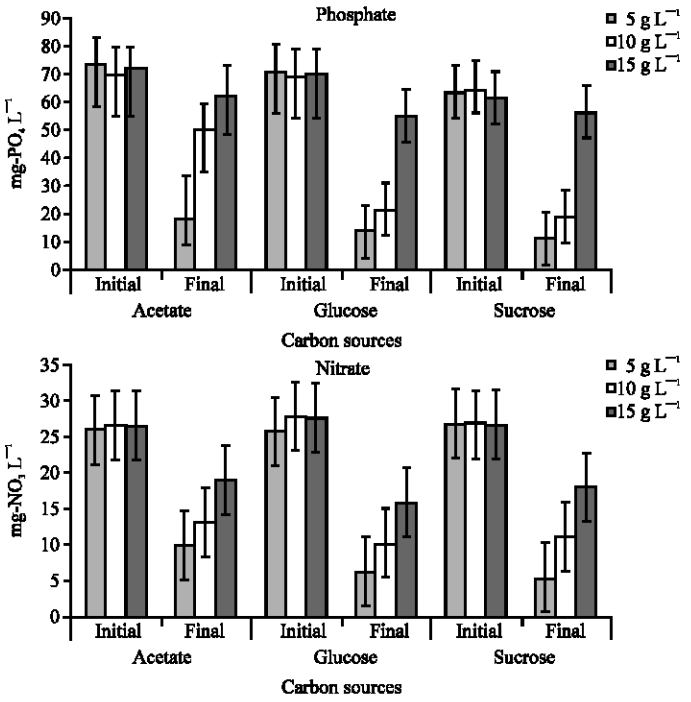

Fig. 2: Phosphate and nitrate uptake by isolate B at different concentrations of the carbon sources (acetate, glucose and sucrose) in mixed liquor. Initial and final means concentration at time zero and after 96 h incubation, respectively

In the presence of acetate, significant variation was only observed between concentration of 5 and 15 g L^{-1}, while in the case of glucose, significant variation was observed between the different concentrations ($p \leq 0.05$). No significant variation was observed between concentrations of 10 g L^{-1} and 15 g L^{-1} in the presence of sucrose.

In mixed liquor containing isolate C, phosphate and nitrate uptakes were observed to be highest at concentrations of 5 g L^{-1} (Fig. 3). This trend was common in the presence of either of the carbon sources. In the presence of acetate, a decrease from 70.50 to 14.59 mg L^{-1} and 26.67 to 13.42 mg L^{-1} were observed for phosphate and nitrate, respectively. Also, a decrease from 71.76 to 13.58 mg L^{-1} and 63.51 to 7.37 mg L^{-1} (for phosphate) and from 25.60 to 7.32 mg L^{-1} and 26.60 to 5.34 mg L^{-1} (for nitrate) were observed in the presence of glucose and sucrose, respectively (Fig. 3).

For phosphate uptake, in the presence of acetate or sucrose, no significant variation was observed between concentration of 5 and 10 g L^{-1}. However, in the presence of glucose, there was a significant variation between concentration of 5 g L^{-1} and either 10 or 15 g L^{-1} ($p \leq 0.05$). A similar trend was also observed in nitrate uptake at the same probability level.

With respect to COD concentration of the mixed liquor, in the presence of acetate as carbon source, there was no observed removal, irrespective of the isolates. COD concentration in presence of acetate was found to be highest at acetate concentration of 15 g L^{-1}; increasing from 464.45 to 1467.88 mg L^{-1}, 498.85 to 1221.32 mg L^{-1} and 418.57 to 1410.54 mg L^{-1}, in mixed liquor containing isolates A, B and C, respectively (Fig. 4-6).

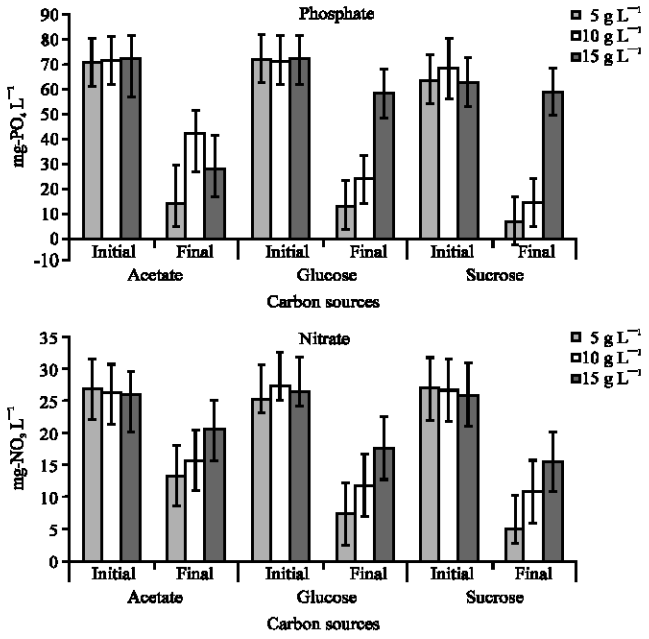

Fig. 3: Phosphate and nitrate uptake by isolate C at different concentrations of the carbon sources (acetate, glucose and sucrose) in mixed liquor. Initial and final means concentration at time zero and after 96 h incubation, respectively

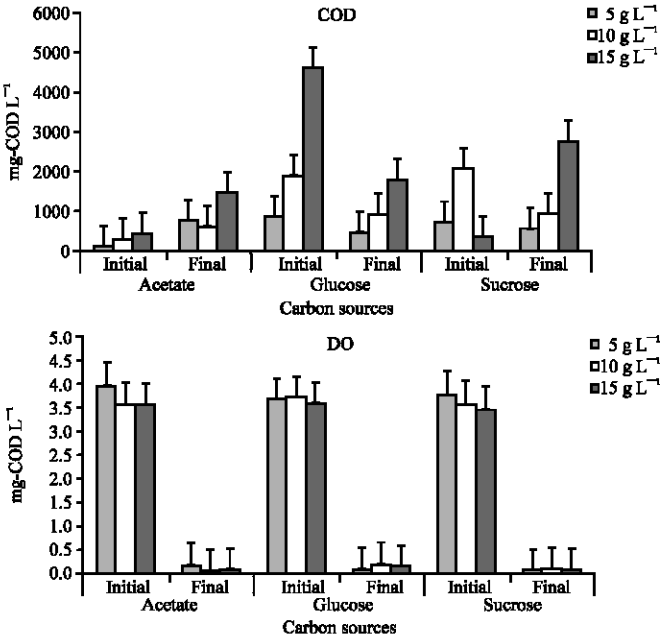

Fig. 4: COD and DO concentrations of mixed liquor inoculated with isolate A in different concentrations of the carbon sources (acetate, glucose and sucrose). Initial and final means concentration at time zero and after 96 h incubation, respectively

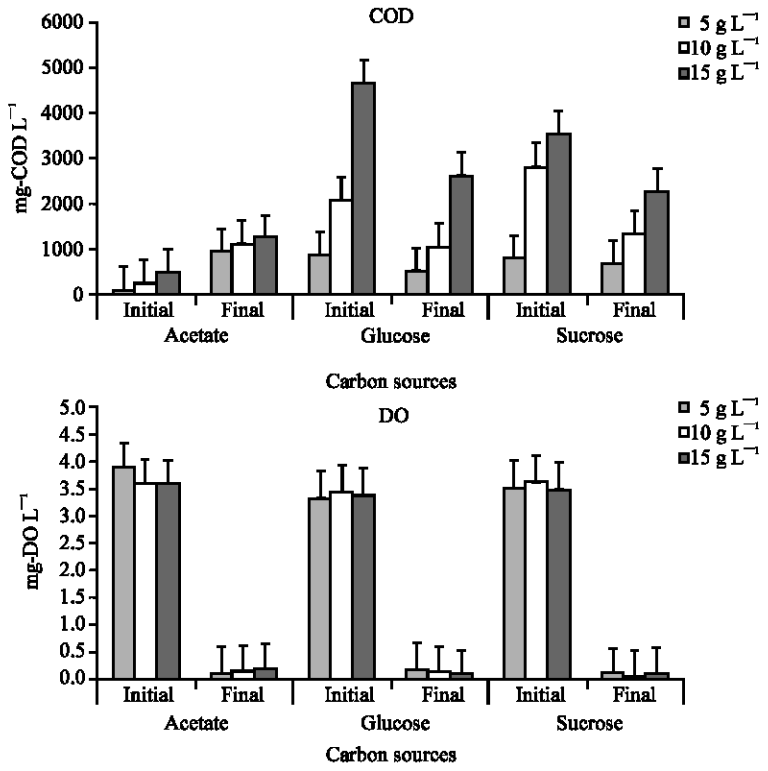

Fig. 5: COD and DO concentrations of mixed liquor inoculated with isolate B in different concentrations of the carbon sources (acetate, glucose and sucrose). Initial and final means concentration at time zero and after 96 h incubation, respectively

In the presence of glucose or sucrose as carbon source, COD removal was observed. Maximum removal was observed at a glucose or sucrose concentration of 5 g L^{-1}. In mixed liquor containing isolates A, B and C COD concentration decreased from 871.55 to 458.71 mg L^{-1}, 885.31 to 516.03 mg L^{-1} and 908.25 to 668.87 mg L^{-1}, respectively in the presence of glucose. In the presence of sucrose COD concentration was observed to decreased from 745.41 to 573.39 mg L^{-1} and 791.28 to 688.07 mg L^{-1} in mixed liquor containing isolates A and B, respectively (Fig. 4-6).

Dissolved oxygen was observed to decrease drastically in mixed liquor at the end of the 96 h incubation. This was irrespective of the isolates or concentration of carbon source present in the mixed liquor (Fig. 4-6). There was no significant difference between the DO concentrations of the mixed liquor among the different concentrations of the carbon sources.

As shown in Fig. 7, at the end of 96 h incubation, growth rate of the isolates in the presence of acetate was observed to be highest at a concentration of 10 mg L^{-1} for isolate A and B and 15 mg L^{-1} in isolate B. In the presence of glucose, growth rate was highest at a concentration of 10 mg L^{-1} for isolate A and 15 mg L^{-1} in isolates B and C. Growth rate was highest at a sucrose concentration of 15 mg L^{-1} (Fig. 7).

Although the growth rates of the isolates at different carbon source concentrations were different, these differences were not observed to be significant ($p \leq 0.05$). This was irrespective of the carbon sources.

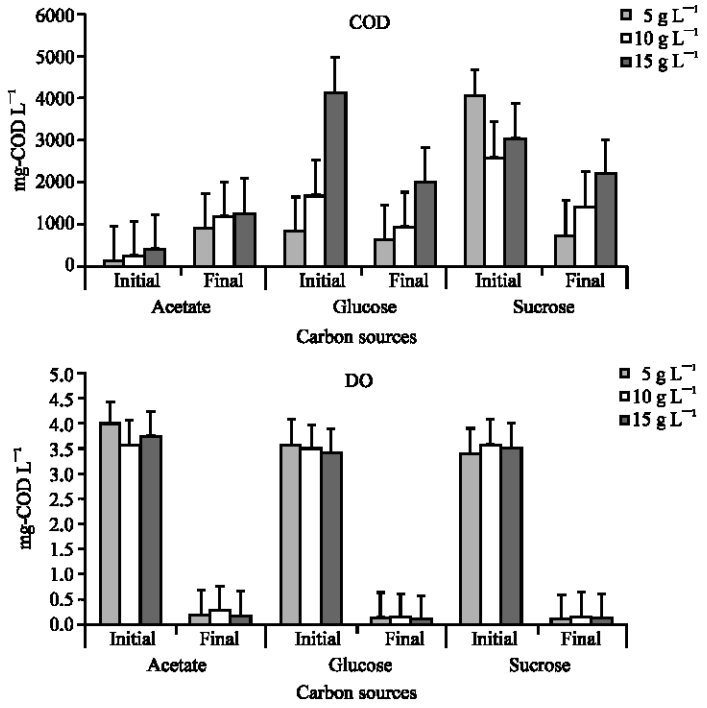

Fig. 6: COD and DO concentrations of mixed liquor inoculated with isolate C in different concentrations of the carbon sources (acetate, glucose and sucrose). Initial and final means concentration at time zero and after 96 h incubation, respectively

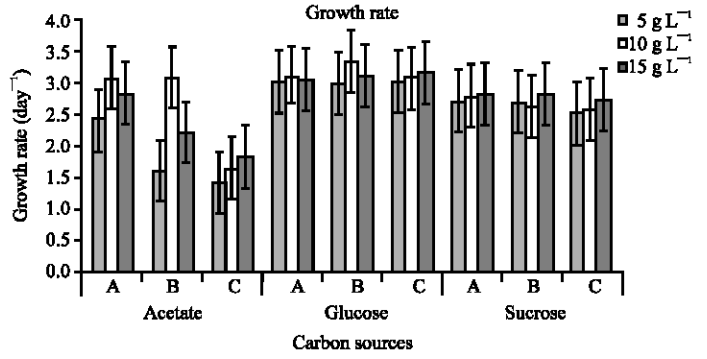

Fig. 7: Growth rate of the isolates in mixed liquor containing different concentrations of carbon sources (acetate, glucose and sucrose) after 96 h of incubation. A, B and C are the protozoan isolates

DISCUSSION

Three carbon sources (acetate, glucose and sucrose) were used for this investigation. The choice of these carbon sources was deliberate. They have been reported to enhance biological removal of nutrients from wastewater (Isaacs and Henze, 1995; Jeon and Parker, 2000). Another reason for choosing these sugars was because of their low cost and presence in industrial wastewaters, thus

making them potential cheap sources of carbon for biological treatment of wastewater with high nutrient contents (Guadalupe *et al*., 1998).

Acetate has been reported to be a preferred carbon source for phosphate removal from wastewaters by luxury uptake organisms (Kargi and Uygur, 2003). Other reports have indicated glucose and other similar carbohydrates as essentials in energy generation, thus improving the performance of sequencing batch reactor operation for nutrient removal (Sang *et al*., 1997; Chang *et al*., 2000).

The present study revealed the fact that with increasing concentration of any of the carbon source used a decrease in either phosphate or nitrate uptake efficiency of the test isolates was observed. This observation may have been due to the presence of excess organic matter in the mixed liquor at such high concentrations of carbon source. Although reports have shown that protozoa live by the direct removal of organic nutrients when bacteria are unavailable, it has further been suggested that substrate compounds in the effluent feed affect the performance of nutrient removal systems (Curds and Cockburn, 1970; Haga *et al*., 1995).

Henze (1989) have reported that nutrient removal is more easily obtained with the most easily degradable carbon forms, hence controlled addition of carbon sources to nutrient removal processes can serve as a means of improving stability and flexibility. Also, although carbon source is known to be important in nutrient removal processes, excess of such carbon source is reported to cause a decrease denitrification. Studies have shown that denitrification is strongly influenced by the nature and quantity of carbon source (Isaacs and Henze, 1995).

The low phosphate and nitrate uptake observed at higher concentrations of carbon source have been reported by Isaacs and Henze (1995). This may likely be as a result of build-up of organics of various degrees, hence a significant concentration of the carbon will need to be first hydrolysed before nutrient uptake can be achieved (Kristensen and Jorgensen, 1990; Heymann, 1985).

In this study, it was observed that at high initial COD (high initial carbon concentration), nutrient uptake was low. A similar finding has been reported by You *et al*. (2004). In their report, they observed that at low initial COD concentration, phosphate uptake was high but major reaction turned into phosphate release at high residual COD.

Although there was minimal nutrient uptake at high initial COD concentration, reports have shown that at increased COD, nutrient uptake rate is decreased (Isaacs and Henze, 1995). Despite the fact that this was not the finding in this investigation, some reports have shown that at low carbon source levels, denitrification is limited, hence there should be sufficient organic carbon sources for proper denitrification (Obaja *et al*., 2005). Henze (1991) has suggested that for processes employing internal carbon, sufficient carbon must be available in order to achieve complete denitrification of the nitrate formed during nitrification.

The protozoa growth rates in different carbon source concentration were not observed to vary significantly in this investigation. We could therefore deduce that the growth rates of the isolates during nutrient uptake were unaffected by the concentration of carbon source used. Although we could not compare our findings on this with other investigations, due to non availability of data on that, it has been reported that during biological wastewater treatment, the active biomasses are responsible for nutrient uptake (Sidat *et al*., 1999).

This study showed DO concentrations of mixed liquor decreasing drastically, irrespective of concentration of carbon source and did not vary significantly at the end of incubation, thus showing that carbon concentration did not have an effect on DO consumption by the test isolates. Initial DO values in all our set up were in a value greater than 3.0 mg L^{-1}. It is reported that activated sludge processes designed for nutrient removal should have DO levels greater than 2.0 mg L^{-1} (Louzeiro *et al*., 2002).

Although this was not investigated in this study, Brdjanovic *et al*. (1996) have revealed that DO concentrations greater than 4 mg L^{-1} do not appear to further stimulate biological nutrient removal, hence excessive aeration tends to negatively affect biological nutrient removal processes.

CONCLUSION

The present study was based on the relationship between 3 carbon source (acetate, glucose and sucrose) concentration and nutrient uptake efficiency of 3 protozoan isolates.

Our findings revealed the fact that increasing concentration of any of the tested carbon sources did not enhance nutrient uptake. This investigation was able to reveal optimum carbon source concentration in mixed liquor for nutrient uptake by the isolates to be 5 g L^{-1}. The study also showed that at increased concentration of acetate, glucose or sucrose, initial COD of mixed liquor was high and nutrient uptake was retarded. Both the growth rates of the isolates and DO concentration of mixed liquor were not observed to be affected by initial carbon source concentration.

This study had therefore been able to give an insight into the optimum acetate, glucose and sucrose concentration in mixed liquor that will enhance nutrient uptake by protozoa, which was not well documented.

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