

Full Length Research Paper

The effects of pH and temperature on phosphate and nitrate uptake by wastewater protozoa

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The objective of this study was to ascertain the effects of pH and temperature on nutrient uptake efficiency of 3 wastewater protozoan isolates (*Aspidisca* (A), *Trachelophyllum* (B) and *Peranema* (C)) that have previously been screened for nutrient uptake ability. The study was carried out in shake flask at pH 5, 6, 7, 8 and 9 and incubation temperatures of 25, 30 and 35°C for 96 h. The results revealed optimum pH ranges for the uptake of phosphate and nitrate to be 7 to 9 and 5 to 7, respectively. Maximum nutrient uptake was found to occur at 25°C with phosphate concentration decreasing from 64.74 to 10.21 mg/l, 63.09 to 8.54 mg/l and 64.47 to 6.36 mg/l, for isolates 'A', 'B' and 'C', respectively. Also, nitrate concentration was found to decrease from 24.71 to 4.91 mg/l for isolate 'A', 24.47 to 11.15 mg/l for isolate 'B', and 24.58 to 15.00 mg/l for isolate 'C' at the same temperature. An increase in COD was observed in mixed liquor inoculated with the test isolates. The study has been able to give an insight into the optimum temperature and pH for phosphate and nitrate uptake by the isolates.

Key words: Nitrate, pH, phosphate, temperature, uptake.

INTRODUCTION

Wastewater may contain high levels of nutrients, which when excessively released to the environment can lead to the undesirable growth of microorganisms and hence eutrophication. The two major eutrophic nutrients present in effluents from wastewater are nitrogen and phosphorus compounds (Dicicco, 1979; Sahset et al., 2006). The presence of these nutrients in wastewater, causes ecological impacts and affect public health, thus the control of their emission into receiving water bodies is therefore essential (Amir et al., 2004; Hosni et al., 2007).

Although biological nutrient removal has been attributed mostly to bacteria, there is now increasing evidence that protozoa play important roles in nutrient recycling in aquatic ecosystems (Coran, 1986; Jacqueline and Barry, 1995). Other studies have reported the involvement of protozoa in enhanced mineralization of nutrients, like carbon, nitrogen and phosphorus in terrestrial environment (Sherr et al., 1983; Anderson and Griffic, 2001). Also, several protozoa have been implicated in the excretion

of mineral nutrients, thus resulting in accelerated use of carbon sources by other organisms.

Despite the fact that protozoa are known to enhance the mineralization of nutrients (phosphorus and nitrogen) in aquatic microcosms and in activated sludge, little is known on the effect of temperature and pH on their nutrient removal efficiency. This then forms the basis of this investigation.

MATERIALS AND METHODS

All chemicals used were of analytical grade. All flasks were incubated in a rotary shaker, at a shaking speed of 100 rpm. Aliquot samples were taken at time zero and every 24 h for the analysis of the above mentioned parameters. The duration of incubation for each experiment setup was 96 h.

Three protozoa (*Aspidisca* (A), *Trachelophyllum* (B) and *Peranema* (C)) were used for this study. They were isolated from the aerobic zone of Daasport wastewater treatment plant in Pretoria, South Africa, between October and November, 2006. The isolates have previously been screened for phosphate and nitrate removal efficiency (Akpor et al., 2007).

In order to evaluate the effect of pH and temperature on biological phosphate and nitrate uptake by the test protozoa, mixed

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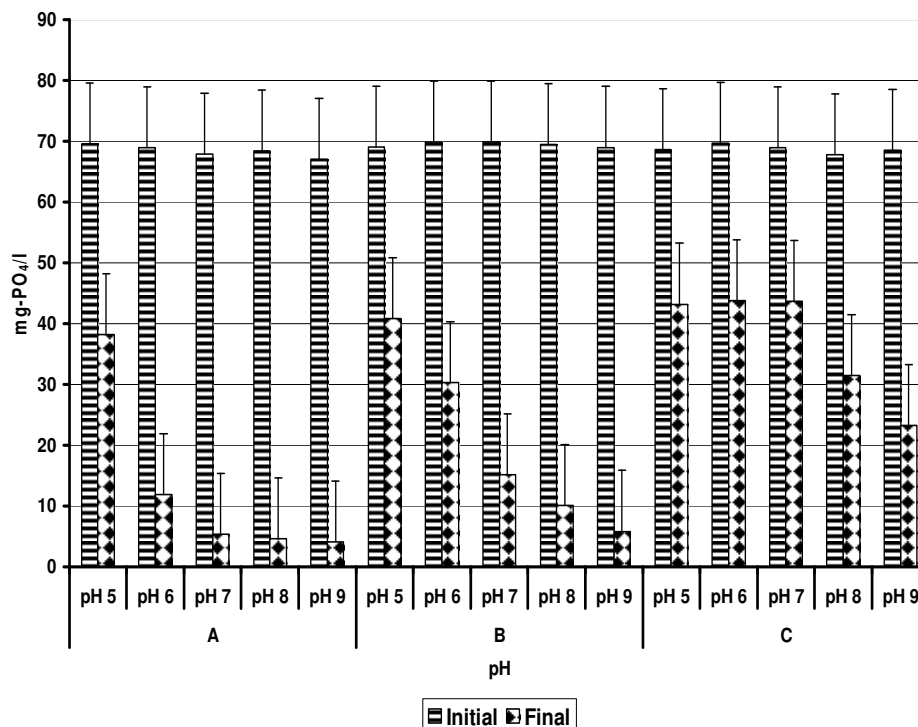


Figure 1. Average phosphate concentrations in mixed liquor inoculated with the isolates at different pH. Initial and final means concentrations at time zero and after 96 h incubation. A, B and C represents the wastewater protozoan isolates, *Aspidisca*, *Trachelophyllum* and *Peranema* species, respectively.

liquor was obtained from the anaerobic zone of Daasport wastewater treatment plant in Pretoria, South Africa. The mixed liquor was filtered, using Whatman No. 1 filter paper and then supplemented with CH_3COONa , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and KNO_3 in concentrations of 5, 0.5, and 0.18 g/l, respectively before sterilization in an autoclave. Prior inoculation with the test protozoa isolates, the following antibiotics were added to the mixed liquor: penicillin (10 $\mu\text{g}/\text{ml}$), streptomycin (66 $\mu\text{g}/\text{ml}$) and tetracycline (100 $\mu\text{g}/\text{ml}$). This was done to inhibit any contamination by bacteria.

The study was performed in two phases: In the first phase, the impact of pH variation; and in the second phase, the influence of temperature variation on nutrient uptake by the test isolates was investigated. Phosphate and nitrate were analyzed, using the ascorbic acid and salicylate methods, respectively, as described in standard methods (APHA, 2001). Chemical oxygen demand (COD) was determined, using the closed-reflux method, as described also in standard methods.

RESULTS AND DISCUSSION

The results of the effect of pH on phosphate uptake by the respective isolates in mixed liquor sample are shown in Figures 1 and 2. As can be seen from Figure 1, optimum pH range for phosphate uptake was found to be from 7 to 9, for isolate 'A' and 'B', and from 8 to 9 for isolate 'C'. However, in all the isolates, the highest uptake was observed at pH 9, reducing from 68.01 to 4.11 mg/l, 68.98 to 5.86 mg/l and 68.50 to 23.25 mg/l, for isolates 'A', 'B' and 'C', respectively.

This result is in conformity with what have been reported by earlier workers. In a study conducted by Sahset et al. (2006), on the effect of pH on phosphate removal from wastewater by electro-coagulation with iron plate electrodes, the highest phosphate removal was observed at a pH range of 7 to 9. A similar result was obtained by Huigiang (2007). However, our result was at variance with the report of Yan et al. (2007). In their report, when the effect of various initial pH values on anaerobic and aerobic transformations of soluble ortho-phosphate were investigated, anaerobic phosphate release decreased when initial pH increased from 6.4 – 6.8, but increased as pH was raised from 6.8 – 8.0. Raising pH values to 8 have been reported to lead to a reduction in phosphate concentration (Zhu et al., 2001).

In the case of nitrate uptake, optimum uptake was observed at a pH of 6 - 7 for isolate 'A', 5 - 8 for isolate 'B' and 5 to 6 for isolate C (Figure 2). In all the 3 isolates, highest uptake of nitrate was noticed at pH 6, decreasing from 17.68 to 3.20 mg/l for isolate 'A', 16.12 to 7.62 mg/l for isolate 'B' and from 17.80 to 7.23 mg/l for isolate 'C'. Although some workers (Zawaideh and Zhang, 1998) have reported that at normal pH ranges, nitrate removal is usually less than 50% without buffer treatment. As stated earlier, an attempt was made to buffer our pH but this was observed not to support the growth of our isolates, therefore there was no result to compare the effect

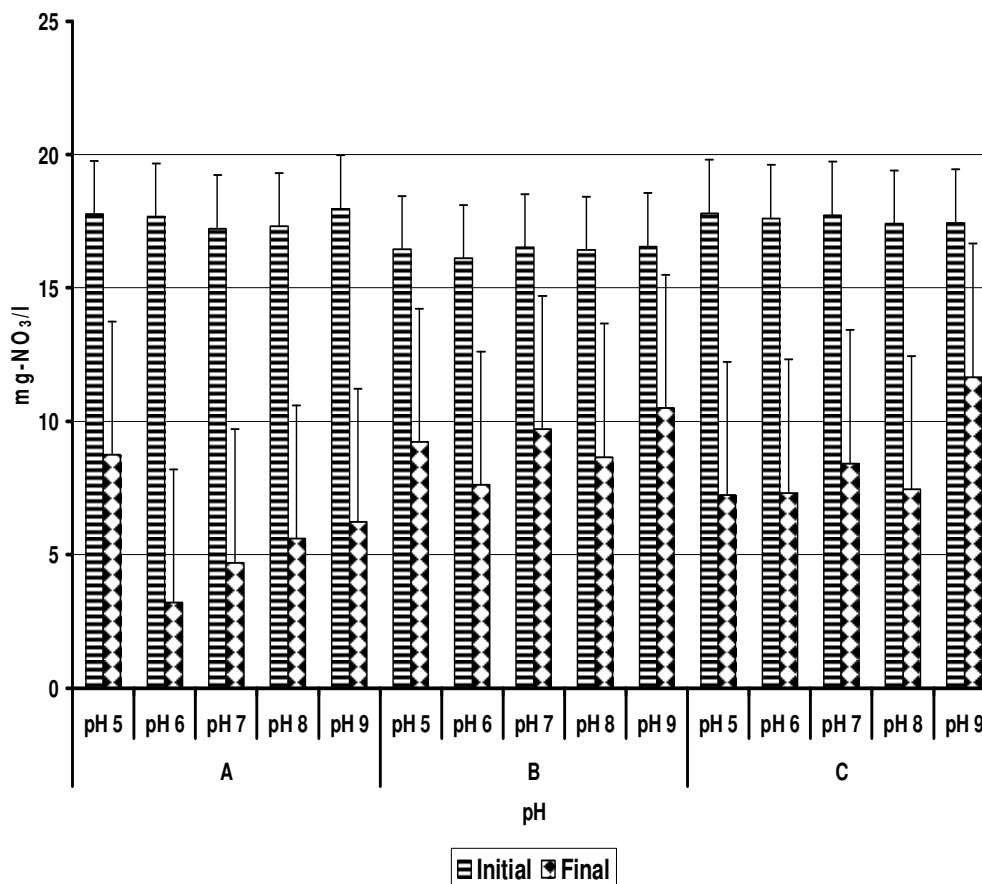


Figure 2. Average nitrate concentrations in mixed liquor inoculated with the isolates at different pH. Initial and final means concentrations at time zero and after 96 h incubation. A, B and C represents the wastewater protozoan isolates, *Aspidisca*, *Trachelophyllum* and *Peranema* species, respectively.

of a buffered pH and a non-buffered pH on nitrate removal.

Nese and Ennil (2004) have reported that the most effective pH for nitrate removal is at 2 for powdered activated carbon but in the presence of other absorbents, pH value did not affect nitrate removal. The acidity or alkalinity of wastewater is known to affect biological treatment. It is reported that the pH of wastewater needs to remain between 6 and 9 to protect organisms. Extremely low or high pH values were not tested in this study; this is because such pH concentrations cannot support the growth of our isolates, of which pH is important. For biological nutrient removal to be accomplished, the environment must support the growth of the microorganisms (Warangkana and Randall, 1997). Acids and other substances that alter pH can inactivate treatment processes when they enter wastewater from industrial or commercial sources (Metcalf and Eddy, 1999).

The effect of temperature on phosphate and nitrate uptake by the test protozoan isolates are shown in

Figures 3 and 4. As shown in the figures, phosphate and nitrate uptake was observed to be optimum at temperature range of 25 to 30°C. Highest uptake was, however observed at 25°C, decreasing (in the case of phosphate uptake) from 64.74 to 10.21 mg/l for isolate 'A', 63.09 to 8.54 mg/l for isolate 'B' and 64.47 to 6.36 mg/l for isolate 'C'. At the same temperature of 25°C, nitrate concentration decreased from 24.7 to 4.91 mg/l for isolate 'A', 24.47 to 11.15 mg/l for isolate 'B' and from 24.58 to 15.00 mg/l for isolate 'C'. At a temperature of 35°C, concentrations of phosphate and nitrate removed were observed to be minimal. This trend was irrespective of isolate.

Temperature has been known to have an effect on growth processes of protozoa. For example, cell volume often decreases with increasing water temperature. It has also been observed that gross growth efficiency, for protozoan species may increase, decrease or remain unchanged in response to increasing water temperature (Caron et al., 1986, Brooks, 1996). In general, biological treatment activity is reported to accelerate in warm temperatures and slows in cool temperatures, but extremely

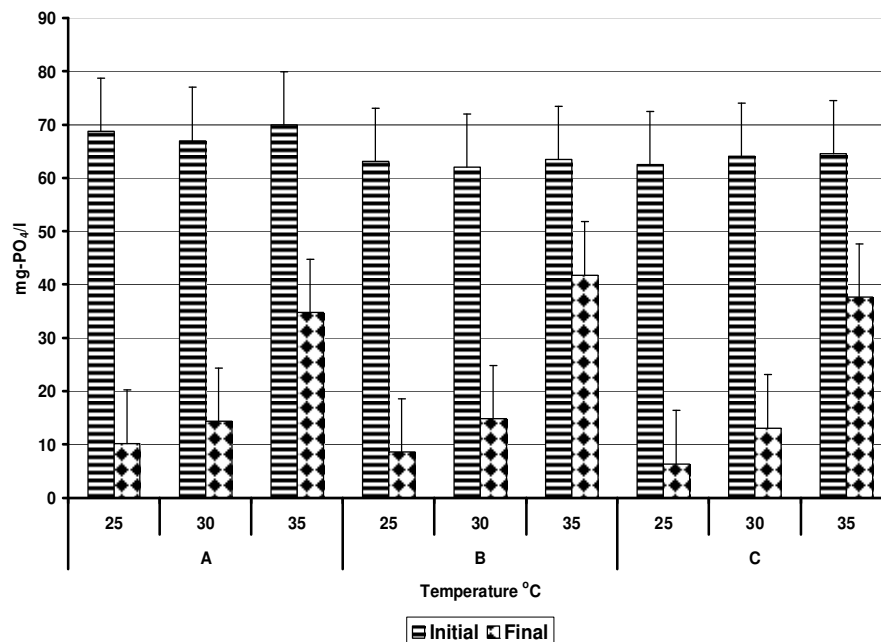


Figure 3. Average phosphate concentrations in mixed liquor inoculated with the isolates at different temperature. Initial and final means concentrations at time zero and after 96 h incubation. A, B and C represents the wastewater protozoan isolates, *Aspidisca*, *Trachelophyllum* and *Peranema* species, respectively.

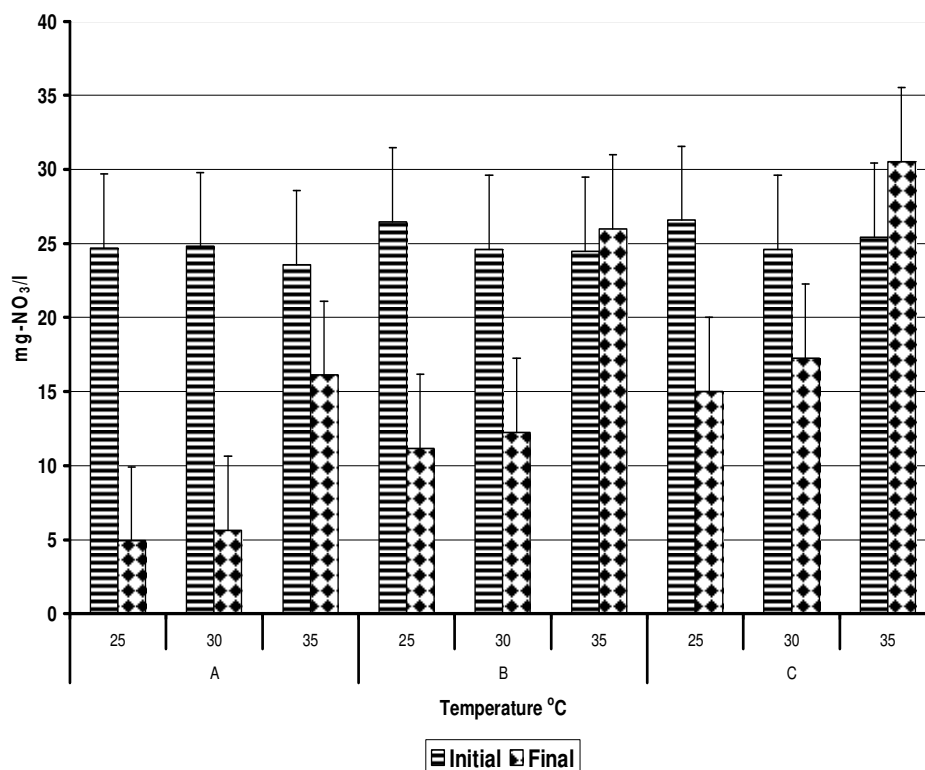


Figure 4. Average nitrate concentrations in mixed liquor inoculated with the isolates at different temperature. Initial and final means concentrations at time zero and after 96 h incubation. A, B and C represents the wastewater protozoan isolates, *Aspidisca*, *Trachelophyllum* and *Peranema* species, respectively.

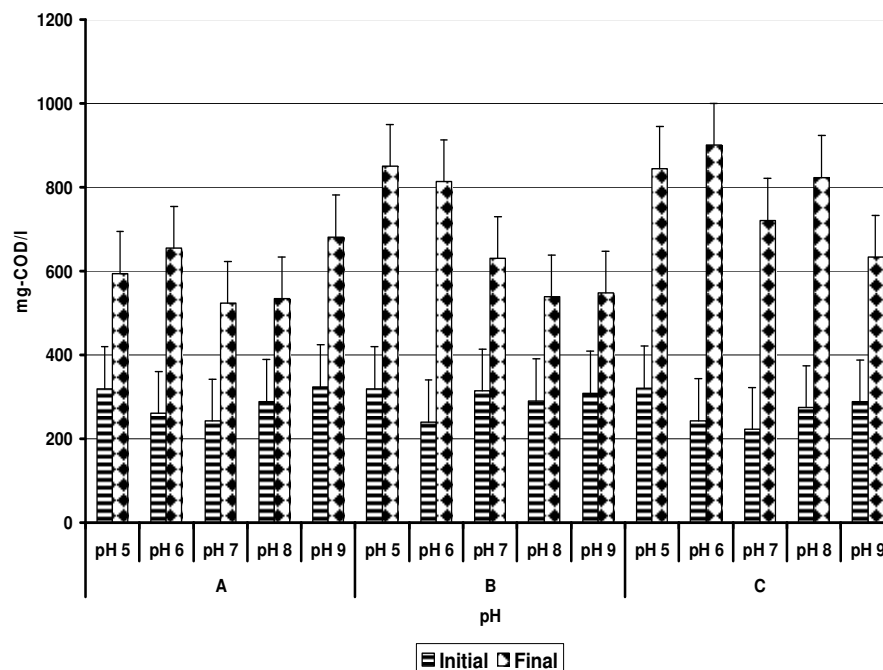


Figure 5. Concentrations of mixed liquor COD at different pH. Initial and final means concentrations at time zero and after 96 h incubation. **A**, **B** and **C** represents the wastewater protozoan isolates, *Aspidisca*, *Trachelophyllum* and *Peranema* species, respectively.

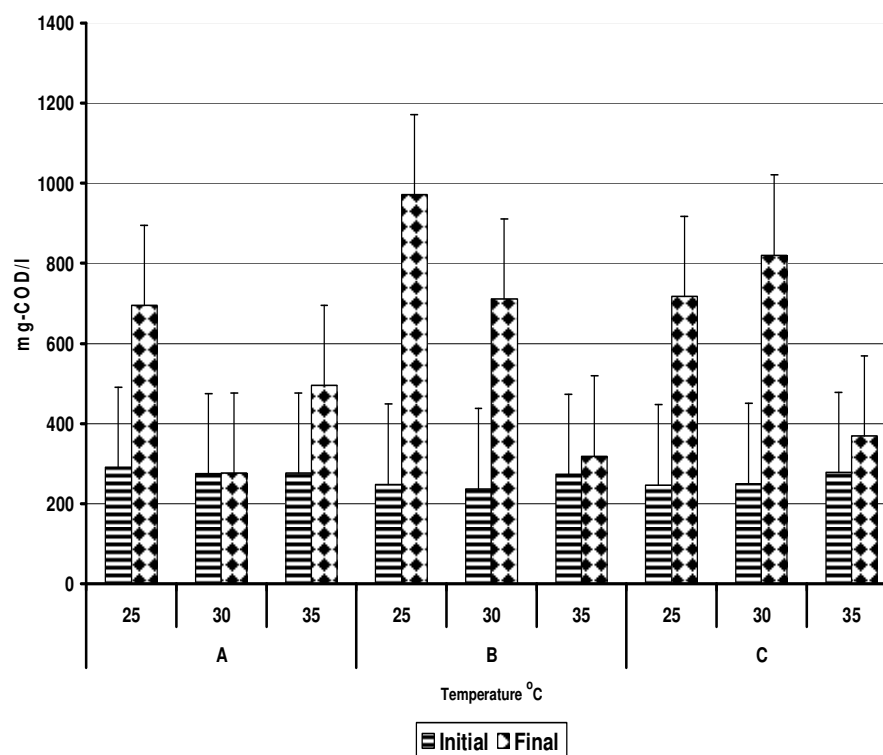


Figure 6. Concentrations of mixed liquor COD at different temperatures. Initial and final means concentrations at time zero and after 96 h incubation. **A**, **B** and **C** represents the wastewater protozoan isolates, *Aspidisca*, *Trachelophyllum* and *Peranema* species, respectively.

hot or cold temperatures can stop treatment processes (Metcalf and Eddy, 1999).

Temperature is also reported to be one of the key parameters that affects the reaction kinetics and performance of biological nutrient removal systems. There are however, conflicting reports on the effect of temperature on enhanced biological nutrient removal systems (Erdal et al., 2003; Thongchai et al., 2003). Marais and Jenkins (1992) have reported that the optimum operating temperature for biological nutrient removal processes should range from 28 to 33°C.

As shown in Figures 5 and 6, COD concentrations in mixed liquor were observed to increase in presence of the test isolates. This was irrespective of pH and incubation temperature. Concentrations of COD in mixed liquor were observed to increase from 261.36 to 654.56 mg/l, 241.15 to 813.80 mg/l and 243.56 to 900.24 mg/l at pH 6, for isolates A, B and C, respectively. In the case of temperature, highest COD increase was observed at 25°C for isolates A and B and at 30°C for isolate C, thus increasing from 290.97 to 694.36 mg/l, 249.06 to 970.95 mg/l and 250.15 to 820.23 mg/l for isolates A, B and C, respectively.

Although some workers (Lee and Welander, 1996) have reported a decrease in COD in similar studies, this was not observed in this study. Ryu et al. (2007), when investigating the effect of pH on COD removal in activated sludge system, revealed that maximum COD removal efficiency is obtained when influent wastewater pH is adjusted to 7.0. This disparity may have been due to the type of wastewater used. In this study, the wastewater used was mixed liquor that was supplemented with sodium acetate salt.

Conclusion

This study has been able to show the optimum pH and temperature for nutrient uptake by the test isolates, which was previously not well known. Although these findings cannot be considered to be exhaustive, as further work is going on to ascertain the effect of other nutrient supplements in the mixed liquor on nutrient removal by the isolates, it has still given an insight to the optimum pH and temperature for nutrient uptake by wastewater protozoa. This knowledge will help in an effective biological wastewater treatment.

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