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ORIGINAL ARTICLE

Effect of nickel on nutrient removal by selected indigenous protozoan species in wastewater systems

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Abstract Nutrient and heavy metal pollutions are major concern worldwide. This study aimed at comparing the effect of Ni^{2+} on nutrient removal efficiency of four indigenous wastewater protozoan species (*Aspidisca* sp., *Paramecium* sp., *Peranema* sp., *Trachelophyllum* sp.). Specific physico-chemical parameters and microbial growth/die-off were measured using standard methods. The results revealed that protozoan species were able to simultaneously remove phosphate, nitrate and Ni^{2+} at concentrations ranging between 66.4–99.36%, 56.19–99.88% and 45.98–85.69%, respectively. *Peranema* sp. appeared to be the isolates with the highest removal of nutrients (Phosphate-99.36% and Nitrate-99.88%) while *Paramecium* sp. showed higher removal of Ni^{2+} at 85.69% and low removal of nutrients. *Aspidisca* sp. was the most sensitive isolate to Ni^{2+} but with significant nutrient removal (Phosphate-66.4% and Nitrate-56.19%) at 10 mg-N^{2+}/L followed by an inhibition of nutrient removal at Ni^{2+} concentration greater than 10 mg/L. Significant correlation between the growth rate and nutrient removal ($r = 0.806/0.799$, $p < 0.05$ for phosphate and nitrate, respectively) was noted. Except for *Peranema* sp. which revealed better nutrient removal ability at 10 mg-Ni^{2+}/L, an increase in Ni^{2+} concentration had a significant effect on nutrient removal efficiency of these indigenous protozoan species. This study suggests that although Ni^{2+} appeared to be toxic to microbial isolates, its effect at a low concentration (10 mg-Ni^{2+}/L) towards these isolates can be used to enhance the wastewater treatment process for the removal of nutrients. *Peranema* sp., which was able to remove both Ni^{2+} and nutrients from wastewater mixed-liquor, can also be used for bioremediation of wastewater systems.

1. Introduction

Since the twentieth century, the world has been characterised by a dramatic increase in the human population, the rising standard of living, industrialisation, mining operations and urbanisation. These features have been reported to negatively impact not only on the use of available water resources, but also on aquatic life. Pollutions caused by chemical pollutants,

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including nutrients and heavy metals, are currently a global issue (Balamane-Zizi and Ait-Amar, 2012).

Nutrient pollution caused by excessive inputs of mostly nitrogen and phosphorus has been reported as being the main contributor to eutrophication in water sources (Boesch, 2002). Nitrogen is the most abundant chemical element in the earth's atmosphere (approximately 80%), and also one of the essential and crucial elements for any form of life (Camargo and Alonso, 2006). Since most living organisms cannot use nitrogen in the gaseous form, ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) are the most common variety of chemical forms present and used in the aquatic environment (Rabalais et al., 2009). Besides this important role, nitrate in groundwater and surface water has been reported to pose a potential health hazard when present in excessive concentrations (Akpor et al., 2008). It has been found that nitrate plays a major role in blue baby syndrome (Knobeloch et al., 2000).

Similar to nitrogen, phosphorus (phosphate) has been recognised as one of the most common elements on earth and an essential nutrient to all living organisms. Previous studies have pointed out that in living organisms, phosphorus plays a crucial role in the transfer and storage of energy (ATP), photosynthesis, the manufacture of nucleic acids, proteins and carbohydrates, etcetera (Rychter and Rao, 2005). However, when its input into the aquatic environment becomes higher, phosphorus leads to a rapid algal growth which results in eutrophication (Akpor et al., 2008). Phosphate toxicity has been reported to accelerate the mammalian ageing process, to compromise the functional ability of various organisms by exerting cytotoxic effects, to provoke several complications such as tetany, dehydration, hypotension, tachycardia, hyperpyrexia, cardiac arrest and coma (Razzaque, 2011). In addition, a higher occurrence of vascular calcification in patients with chronic kidney disease and pulmonary oedema has been reported as a common consequence of phosphate toxicity (Osuka and Razzaque, 2012).

When compared to nitrogen, phosphorus is regarded as a major concern in water pollution since cyanobacteria, mainly responsible for eutrophication, are capable of fixing molecular nitrogen from the atmosphere, thus eliminating the requirement for ammonia (NH_3-N) or nitrate (NO_3-N) (Lopez-Vazquez, 2009). In their study, Korstee and co-workers Korstee et al. (1994)) found that the phosphorus concentrations of 8–10 ug/L, even at nitrogen concentrations of 4–5 mg/L, resulted in the hindrance of the phenomenon of eutrophication. To counteract this phenomenon, several conventional wastewater treatment methods have been developed for the removal of both nitrogen and phosphorus (Akpor et al., 2008). These include chemical oxidation, chemical precipitation, ion exchange, etc. Due to their advantages over the conventional methods, biological treatments have been widely used to treat wastewater containing high nutrient concentrations (Wagner et al., 2002). Microorganisms are therefore known to play an active role in the solubilisation, accumulation, transport and deposition of pollutants in the environment (Cheremisinoff, 1995; Atuanya and Oseghe, 2006). Although the dynamic population of wastewater systems includes bacteria, fungi, rotifers, viruses, nematodes, and protozoa, less attention has been focused on protozoan species when compared to algae, fungi and bacteria in terms of the removal of pollutants.

Though the biological treatment methods have been presented as the best means of removing pollutants such as nitrogen and phosphorus, many factors including pH, temperature, toxic heavy metals, dissolved oxygen, and so forth, can affect the efficiency of this method (Knoetze et al., 1980; Tyagi and Couillard, 2009). Chen et al. (2008) reported that heavy metal toxicity is one of the major causes of upset or failure in the biological wastewater treatment process. Boswell et al. (1999) also added that the toxicity of heavy metals could affect cellular metabolism and Polyphosphorus release/uptake during wastewater treatment process. According to Jin et al. (1998), chromium, iron, cobalt, copper, zinc, cadmium, and nickel are the heavy metals identified to be of particular concern in the wastewater treatment process due to their toxicity. Despite the fact that nickel toxic effects on bacterial ability to remove nitrate have been reported previously (Awasthi and Rai, 2005), little is known regarding the effects of nickel toxicity on the protozoan ability to simultaneously remove phosphate and nitrate in wastewater. The present study aimed at assessing the effect of Ni^{2+} on phosphate and nitrate removal as well as the co-removal of Ni^{2+} by selected protozoan isolates.

2. Materials and methods

2.1. Test organisms

The indigenous protozoan species used in this study included *Aspidisca* sp., *Paramecium* sp., *Trachelophyllum* sp. and *Peranema* sp. These protozoan species were isolated from wastewater mixed liquors collected from the aeration tanks of the Daspoort Wastewater Treatment Plant (Pretoria, South Africa). The isolation of the protozoan species was done using an inverted microscope (Axiovert S100, Carl Zeiss) under ×100 to ×400 magnifications and directly picking the isolates with a handmade glass capillary. Furthermore, the protozoan isolates were washed five times using sterile distilled water and transferred to microtitre plates containing 3–5 mL modified Chalkley's medium supplemented with the following antibiotics: penicillin (10 μg/mL), streptomycin (66 μg/mL), tetracycline (100 μg/mL) and sulfamethoxazole (19 μg/mL) to suppress growth of natural bacterial contaminants (Akpor et al., 2008). They have demonstrated the ability to successfully remove nitrate and phosphorus in modified mixed liquor media (Akpor et al., 2008) and also to tolerate V^{5+} and Ni^{2+} separately (Kamika and Momba, 2011, 2012). The preparation of these protozoan species was carried out according to Akpor et al. (2008). Briefly, each protozoan isolate was separately and aseptically transferred from the stock culture to a 500 ml Erlenmeyer containing 100 ml of fresh media of Proteose Peptone Glucose medium (PPG). An antibiotic (streptomycin-50 μg/ml) to prevent bacterial contamination and a heat-killed *Escherichia coli*-WG4 as the source of nutrient were also added. To obtain the needed protozoan concentrations, the inoculated flasks were incubated at room temperature (25 °C) in dark conditions and the cell numbers were determined every hour using a light microscope (Leica DMLS, Type: 020–518.500) at ×100 to ×400 magnifications. Protozoan cells were fixed on the slides using the air-dried techniques and stained with Bismarck Brown Y prior to visualising through the microscope.