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Comparing the tolerance limits of selected bacterial and protozoan species to nickel in wastewater systems

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ABSTRACT

Heavy-metal resistant microorganisms play a significant role in the treatment of industrial wastewater. The detoxifying ability of these resistant microorganisms can be manipulated for bioremediation of heavy metals in wastewater systems. This study aimed at comparing the tolerance limit of selected wastewater protozoan species (*Aspidisca* sp., *Trachelophyllum* sp. and *Peranema* sp.) against Ni^{2+} with that of selected bacterial species (*Bacillus licheniformis*-ATCC12759, *Brevibacillus laterosporus*-ATCC64 and *Pseudomonas putida*-ATCC31483) commonly found in wastewater systems. The isolates were exposed to various concentrations of Ni^{2+} in mixed liquor and their tolerance to Ni^{2+} assessed at different temperatures (25 °C, 30 °C, 35 °C and 40 °C) and pHs (4, 6, 7, 8 and 10). The physicochemical parameters such as chemical oxygen demand (COD) and dissolved oxygen (DO) of the media and the growth rates of the isolates were measured using standard methods. In terms of their minimum inhibitory concentrations (MIC), the results revealed that the isolates could tolerate Ni^{2+} at concentrations ranging between 32 and 52 ppm for protozoa and between 52 and 84 ppm for bacteria. *B. licheniformis*-ATCC12759 was the most tolerant bacterial species (MIC: 84 ppm-Ni^{2+}) while *Peranema* sp. was the most tolerant protozoan species (MIC: 52 ppm-Ni^{2+}). At 10 and/or 20 ppm-Ni^{2+} the growth of *B. licheniformis*-ATCC12759 (6.30 $days^{-1}$ for 10 and 5.73 $days^{-1}$ for 20 ppm-Ni^{2+}), *P. putida*-ATCC31483 (6.02 $days^{-1}$ for 10 and 5.31 $days^{-1}$ for 20 ppm-Ni^{2+}) and *Peranema* sp. (2.15 $days^{-1}$ for 10 ppm-Ni^{2+}) was stimulated after one day of incubation. Statistical evidence showed significant differences ($p = 0.0065$) between the MIC of the six isolates and positive correlations between COD and the growth rates of isolates ($r = 0.8999/0.8810$ for bacteria/protozoa). The tolerance limit of all isolates was significantly dependent on the pH and the temperature. The study suggests that these isolates can be used for the bioremediation of nickel in industrial wastewater systems.

1. Introduction

Since water quality has become an integral part of environmental management today, wastewater treatment processes are intended to achieve a wastewater effluent of acceptable quality (Curds and Cockburn, 1970; Madoni et al., 1996). The main goal of wastewater treatment is to remove as many of the disease-causing agents and chemical pollutants as possible, in order to minimize the risks to public health (Shaler and Klecka, 1986; Akpor et al., 2008). Among the chemical pollutants present in wastewater systems, heavy metals are persistent during wastewater treatment and their toxicity has been reported elsewhere (Nies, 1999; Duncan et al., 2003).

It has been reported that nickel is one of the heavy metals with toxic effects on human health and on the environment (Ainsworth et al., 1980; Madoni, 2000; Arican and Yetis, 2003; Ong et al., 2004; Baytak and Turker, 2005; Shirdam et al., 2006). The average daily intake of nickel from food stuffs for an adult has been estimated to be approximately 152 μg and soluble nickel is associated with an increased risk of cancer to lungs and nasal passages in electrolysis workers with estimated exposure of 1 to 2 ppm (IARC, 1990). South Africa is rated as the largest producer of nickel in Africa (Vermaak, 1997) and nickel is thought to be one of the most mobile heavy metals found in the natural environment in South Africa (Christensen et al., 1996). It is, therefore, crucial to control its release from wastewater discharges into the environment, in order to prevent water pollution that may result in a public health hazard.

A number of techniques have been developed in the past for metal removal and detoxification of wastewater. These include chemical precipitation, chemical oxidation, ion exchange, etc. Since these techniques have shown a lot of disadvantages such as increasing the volume of sludge produced and often resulting in sludge with poor settling and enhancing characteristics, biological treatment using microorganisms is now preferred (Duncan et al., 2003). The dynamic population of wastewater systems, which include bacteria, fungi, rotifers, viruses, nematodes, and protozoa, is the key component in the biological cycling of elements. In the last three decades, interest in the interactions of heavy metals with microorganisms has reached its apogee. These micro-fauna are, therefore, known to play an active role in the solubilization, accumulation, transport and deposition of pollutants such as heavy metals in the environment

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(Maeda and Azumi, 1982; Cheremisinoff, 1995; Atuanya and Oseghe, 2006).

Although some heavy metals are essential elements for both prokaryotic and eukaryotic organisms, they also have a comprehensively toxic effect on cells, even at moderate concentrations, thereby having a noxious effect on the growth and survival of microorganisms (Gikas, 2007; Gikas, 2008). They tend to affect the metabolic functions of microorganisms and in turn reduce the effectiveness of the biological processes in wastewater treatment plants (Madoni et al., 1996; Moten and Rehman, 1998). The toxicity of these heavy metals in wastewater systems depends upon two main factors, namely, metal type and metal concentration. Other factors such as pH, microorganism number, type and strength of the influent are also recorded to affect the toxicity of metals, though to a lesser degree (Van Nostrand et al., 2005).

Among the microorganisms present in the ecosystem, bacteria, fungi and protozoa are generally the first category to be exposed to heavy metal toxicities and serve as very constructive models for investigating toxic effects of metals at the cellular level (Avery, 2001). Furthermore, the acute toxicity of metals to bacteria, fungi and protozoa inhibits their growth and reproduction in wastewater treatment plants and consequently results in the reduction in both cell density and species richness (Babich and Stotzky, 1982; Cheremisinoff, 1995) and oxygen consumption (Slabbert and Grabow, 1986).

Even though several investigations reported the toxicity of nickel to microorganisms such as *Arthrobacter* spp., *Ralstonia* spp. (CH34) and related bacteria (Schmidt and Schlegel, 1989; Liesegang et al., 1993; Rehman et al., 2010), nickel however, in trace element, is seen as essential for the chemilithotrophic growth of certain microbial species (Tabillion et al., 1980) and a determinant for the growth of cyanobacterium *Oscillatoria* sp. (Van Baalen and O'Donnell, 1978).

Several studies have shown that some microorganisms can adapt to environments with moderate concentrations of heavy metals through the acquisition of specific resistance systems which can play a significant role in wastewater treatment systems (Madoni et al., 1996; Gosh et al., 1997; Rajbanshi, 2008; Ezzouhri et al., 2009). Different species of *Aspergillus*, *Pseudomonas*, *Sporophyticus*, *Bacillus*, *Phanerochaete*, etc., have been reported as resistant to heavy metals such as chromium, cadmium and nickel (Rajbanshi, 2008; Rehman et al., 2010). It has been argued that some microorganisms evolve metal-resistance because of their exposure to toxic metals shortly after life began, while others evolved metal-resistance due to recent exposure to metal pollution over the past 50 years (Ji and Silver, 1992; Roane and Peper, 2000). An example of *Pseudomonas* that has been reported to be able to resist in the presence of vanadium and also use it as electron acceptor (Ortiz-Bernad et al., 2004; Rehder, 2008).

Despite the fact that several microorganisms are known to participate in the detoxification process of wastewater systems and successfully used in the production of effluent of high quality (Yan, 2001), few studies have demonstrated the role of protozoa in wastewater treatment and their tolerance to and, therefore, use in the removal of heavy metals needs further investigation (Madoni et al., 1996; Shakoori et al., 2004; Rehman et al., 2005). The purpose of this study, therefore, was to compare the tolerance limits of selected bacteria with those of selected protozoan isolates against varying concentrations of nickel in wastewater samples in order to determine which group of organisms might play a bigger role in the removal of nickel even at its high concentration in wastewater treatment systems. In this study, the effectiveness of the selected microorganisms in the detoxification process of the contaminants was conducted in laboratory-scale reactors which were operated in batches.

2. Materials and methods

2.1. Sample collection and preparation of the culture medium

Wastewater samples were collected on a monthly basis between August and October 2010 from the effluent (before disinfection) of the Daspoort wastewater treatment plant in Pretoria. To remove biomass and other suspended solids, samples were allowed to settle for 2 h prior to filtration (using Whatman No. 1 filter papers). The profile of the filtered samples was determined in terms of the chemical oxygen demand (COD), dissolved oxygen (DO), pH and nickel (Ni^{2+}). The COD concentration was measured using closed reflux methods as described in standard methods (APHA, 2001), while the nickel concentration was determined using the Inductively Couple Plasma Optical Emission Spectrometer (ICP-OES). Other parameters, such as pH and DO were analyzed using a pH probe (Model: PHC101, HACH) and DO probe (Model: LDO, HACH), respectively. D-glucose anhydrate (2.5 g/l), $MgSO_4 \cdot 7H_2O$ (0.5 g/l) and KNO_3 (0.18 g/l) were added to the filtrate to serve as a carbon source and nutrient supplement in the mixed liquor (Momba and Cloete, 1996; Akpor et al., 2008). The experimental study was performed in triplicate for each sample.

The test metal, [$Ni(NO_3)_2$], used in the experimental study was analytical-grade and was purchased from Merck (Pretoria, South Africa). The stock solution of Ni^{2+} at the concentration 1000 ppm was prepared in the deionized water.

From this solution, aliquots of specific volume corresponding to the final Ni^{2+} concentration of the working solution were added to a 350 ml flask containing wastewater mixed liquor medium to obtain a final volume of 150 ml and the pH was adjusted at 7, 2 ± 0.3 using 1.0 M HCl and 1.0 M NaOH (Merck, SA). ICP-OES was used to confirm the metal concentrations in the wastewater mixed liquor medium. The culture medium was autoclaved at 121 °C for 15 min and cooled down to room temperature before use. To check the sterility of this medium, 1 ml aliquot was plated onto the sterile bacteriological agar and incubated at 37 °C for 24 h. Only flasks containing the sterile media were inoculated with a known population of the respective test organism isolates.

2.2. Growth curves of test organisms

To assess the behavior of each test organism in the presence of Ni^{2+}, their growth performance was firstly determined in a nickel-free medium before using for the Ni^{2+} tolerance experimental study. This part of the study was therefore performed under a laminar flow cabinet (Scientific, Model: 807 6FT Horizontal, SA).

2.2.1. Bacterial species

Three bacterial species *Bacillus licheniformis*-ATCC12759, *Brevibacillus lactosporus*-ATCC64 and *Pseudomonas putida*-ATCC31483 were provided by Quantum Biotechnologies (Strydompark Randburg, South Africa). An aliquot of each of these organisms was separately inoculated in 100 ml sterile nutrient broth (NB) in aseptic conditions and incubated at 30 °C (Fonseca et al., 2011) except for *B. licheniformis* which was incubated at 50 °C overnight (Emptage et al., 2009). The growth of bacterial isolates was determined using the spread plate method after dilution (APHA, 2001) on a daily basis for 10 days. Briefly, 100 µl of the diluted NB culture was transferred to Mannitol Egg Yolk Polymyxin (MYP) agar, nutrient agar (NA) and pseudomonas isolation agar (PIA) for *B. licheniformis*, *B. laterosporus* and *P. putida*, respectively. The plates were incubated at 50 °C for *Bacillus* and at 30 °C for the two other bacterial isolates.

2.2.2. Protozoan species

The three protozoan species (*Aspidisca* sp., *Trachelophyllum* sp. and *Peranema* sp.) were obtained from TUT Water Research Group laboratory stock cultures (Tshwane University of Technology, Pretoria, South Africa). They were previously isolated from wastewater mixed liquors collected from the aeration tanks of the Daspoort wastewater treatment plant (Pretoria, South Africa). These protozoan species have proved their ability to remove nitrate and phosphorus successfully in a study conducted in our laboratory by Akpor et al. (2008). During the study period, each type of protozoan species was