Effect of *Artemia franciscana* on the Removal of Nickel by Bioaccumulation

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The present study evaluates the feasibility of using *Artemia franciscana* in reducing the Ni concentration of synthetic wastewater by the process of bioaccumulation. Metallothionein protein plays a key role in the uptake of nickel by *Artemia*. *Artemia* (Brine shrimp) was exposed to an initial nickel concentration of 40 mg/L. Gradual decrease of nickel was observed from 40 mg/L to 5 mg/L with a removal efficiency of 87.5%. The number of organisms were varied to determine the number for the maximum removal efficiency. Metallothionein protein in *Artemia* was estimated by the silver saturation method. The physical parameters such as pH were maintained in an alkaline condition of 9-10, temperature was maintained at room temperature and salinity at 30 -35%. These were found to be the optimal conditions for the survival and reduction of nickel by *Artemia*.

Key words : *Artemia* / Nickel / Metallothionein / Bioaccumulation / Synthetic wastewater.

INTRODUCTION

Heavy metals are elements with atomic weights between 63.5 and 200.6, and a specific gravity greater than 5.0 (Srivastava and Majumder, 2008). With the rapid development of industries such as metal plating, mining and tanneries, as well as factories producing batteries, paper, fertilizer and pesticides, etc., wastewater containing heavy metals is increasingly directly or indirectly discharged into the environment, specially in developing countries. Unlike organic contaminants, heavy metals are not biodegradable and tend to accumulate in living organisms, and many heavy metal ions are known to be toxic or carcinogenic. Metals that are deposited in the aquatic environment may accumulate in aquatic species and in the food chain and cause ecological damage and also posing a threat to human health due to biomagnifications over time (Soegianto and Irawan, 2008; Hafezieh, 2003). Toxic heavy metals of particular concern in the treatment of industrial wastewater include zinc, copper, nickel, mercury, cadmium, lead and chromium. Nickel in amounts exceeding its critical level might bring about serious lung and kidney problems aside from gastrointestinal distress, pulmonary fibrosis and skin dermatitis (Borba et al., 2006).

*Artemia* (Brine shrimp) is a lower crustacean form and has been widely used since the early 1970s as a test organism in short-term toxicity testing for environmental and pharmacological purposes (Personne et al., 1980; Sorgeloos et al., 1980). It has broad tolerance to the environmental factors such as salinity, temperature and dissolved oxygen in the water (Green et al., 2005). This organism possesses an uncommon adaptability to extreme conditions and is found in environments where other life forms cannot be sustained (Triantaphyllidis et al., 1998). *Artemia*, like many other similar organisms, is able to bioaccumulate quite large amounts of elements from the aquatic environment even when their concentrations are extremely low (Petrucci et al., 1995). The habitats in which the genus *Artemia* is found are characterized by the absence of predatory animal species. Therefore, in such environments the evolution of *Artemia* populations has been supported by the abundance of bacteria, protozoa and algae that are the basis of the *Artemia* diet (Amat, 1985). Metallothionein (MT) protein plays

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an important role in the reduction of nickel by Artemia. MTSs are a superfamily of lower molecular mass metal thiolate cluster proteins, with an ability to bind metallic ions (Kagi, 1993; Kojima, 1991).

Bioaccumulation is a fundamental process in environmental toxicology and risk assessment, as it determines the internal dose of potential toxicants (Luoma and Rainbow, 2005). In this present investigation, Artemia was used as a bioaccumulator in reducing the concentration of nickel from the synthetic wastewater. The effect of physical parameters such as pH and salinity was observed in the reduction of nickel. The uptake capacity of the Artemia was found to be increased with the help of the MTSs protein.

MATERIAL AND METHODS

Collection, processing and hatching of Artemia cysts

Artemia cysts were collected from the salt pans located at Kelambakkam, 12°47′N 80° South India. The cysts were processed and hatching of the cysts was carried out in the sea water with the salinity of 35% at a pH of 8.0–8.5. Exactly 1.00 grams of Artemia Cysts were measured out for testing. The viable cysts located at Kelambakkam, 12 cysts were processed and hatching of the cysts was within a 24-hr time period. 10-day old Artemia was used for the experimental study.

Preparation of synthetic wastewater

The Ni stock solution was prepared by dissolving accurately weighed Nickel II chloride in synthetic seawater. The experimental solutions were obtained by diluting the stock solution in accurate proportions to initial concentrations. The Ni concentration chosen for the experimental conditions was 40 mg/L.

Effect of population of organisms

The population of organisms varied from 300–700 per 1000 mL of synthetic wastewater for the experimental study.

Effect of salinity and pH

Artemia was introduced into the synthetically prepared wastewater solution with a various salinity in the range of 30–50% and pH in the range of 8–14. Experimental studies were carried out at room temperature. The synthetic wastewater was analyzed in the Atomic Absorption Spectrophotometer (Shimadzu, AA6300) for the reduction of nickel before and after treatment.

Estimation of MT protein in Artemia

For MT estimation, samples were weighed and placed in a homogenizing tube with a solution of 0.25 M sucrose, and the mixture was homogenized with a motor-driven Teflon pestle at 4°C. The homogenate was centrifuged at 20,000 g for 20 min at 4°C. Supernatant aliquots of 750 µL were analyzed for MT content by the silver-saturation method (Scheuhammer and Cherian, 1986) with small modifications. Samples were incubated with 1ml of 20 mg/L silver solution for 15 min. at 20°C to saturate the metal binding sites of MT. Excess metal was removed by the addition of 200 µL human red blood cell hemolysate to the assay tubes followed by heat treatment in a water bath (100°C for 2 min.). The heat treatment caused precipitation of Ag⁺ bound hemoglobin and other proteins, except for MT which is heat stable. The denatured proteins were removed by centrifugation at 1,000 g for 5 min. The processes of hemolysate addition, heat treatment and centrifugation were repeated three times for each sample. The amount of Ag⁺ in the final supernatant fraction was proportional to the amount of MT present. Silver concentrations were estimated in an atomic absorption spectrophotometer. In a similar way, nickel from the supernatant fraction obtained from the homogenate was estimated by Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Optimal number of organisms

The population of organisms varied from 300, 400, 500, 600 and 700 per 1000 mL of synthetic wastewater. Among these, the number of 500 was found to be very effective in the removal efficiency (Fig.1). 300 and 400 organisms were not very effective in the removal efficiency due to their smaller number, whereas 600 and 700 organisms were also not effective due to their high number, which led to the suffocation of the organisms. Thus, in the present study, the number of 500 organisms was found to be the optimal number for the removal of nickel at the pH of 9–10 and salinity of 30–35%.

Optimal salinity and pH

The maximum reduction of nickel was observed under the salinity conditions of 30–35%. The increasing salinity concentration decreased the reduction of nickel. At 30–35%, the reduction of nickel percentage was found to be 87.5–90%, at the pH of 9–10 with 500 organisms whereas at the salinity conditions of 40, 45 & 50 %, the reduction percentage of nickel was found to be 70, 62.5 and 55%, respectively (Fig. 2). The optimal pH for the present study was found to be 9–10, at the salinity of 30–35% with 500 organisms, which brought out the reduction of 87.5–90%, whereas in other pH conditions, the removal efficiency was found to be lower...
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FIG. 1. Effect of population of organisms on the removal of nickel (pH 9-10; salinity 30–35%; No multiplication takes place).

FIG. 2. Effect of salinity on the removal of nickel (pH 9-10; Number of organisms is 500; No multiplication takes place).

FIG. 3. Effect of pH on the removal of nickel (salinity 30–35%; Number of organisms is 500; No multiplication takes place).
In the present investigation, the higher pH and salinity affected the survival of the organisms. However, at the pH of 9–10 and salinity of 30–35%, maximum survival was observed and it was found to be the optimal condition for removal efficiency.

**Effect of Artemia on the reduction of nickel**

*Artemia franciscana* was found to reduce 87.5% of nickel at the pH of 9–10 and salinity of 30–35%. 500 organisms were used for this experimental study under the optimal pH and salinity conditions. The initial concentration of 40 mg/L nickel was reduced to the final concentration of 5 mg/L on the 5th day (Fig. 4).

Studies on the bioaccumulation of Ni in *Artemia* at the light microscopic level revealed clear morphological variations between the control and experimental *Artemia*. Accumulation of the metal by experimental *Artemia* was evident in the dumping of the particulate matter over the yolk reserve in the gut by the process of diffusion (Figs. 5a, 5b & 5c). Accumulators are creatures that store the metals in a non-toxic state in high amounts. These creatures change the metals somehow to a non-toxic form and store them by granulating them and combining them with metallothionein.

MTs are a class of low-molecular-weight, cytoplasmic, metal-binding proteins that have a high affinity for various toxic heavy metals. MT content in *Artemia* increased in a time-dependent fashion. MT synthesis in *Artemia* is very high and one of the reasons for the high resistance of this organism to pollutants is attributed to this. Elevated levels of such proteins have been suggested as indicating involvement in the uptake, storage, transport and elimination of toxic metals and in the routine metabolism of metal (Del Ramo et al., 1995).

In the present study, MT proteins were found to be one of the reasons for the bioaccumulation of nickel in the *Artemia*. An increase in the MT content was observed in a time-dependent fashion. Increased MT content at 480, 570 and 610 could be observed on the 3rd, 4th and 5th day, respectively, and it was much less in the first two days of exposure (Fig. 6). This increased MT content on 5th day seemed to be responsible for the accumulation of nickel in *Artemia*, which resulted in 87.5% of nickel reduction.

*Artemia* has been reported as an organism tolerant to acute cadmium exposure, with an LC50 that ranges from 93.3 to 280 mg/L (Sarabia et al., 2002). MT synthesis and metal binding are among the mechanisms presumably involved in cadmium tolerance (Martnez et al., 1991). In the present study, MT synthesis and metal binding are among the mechanisms presumably involved in cadmium tolerance.
similarly plays a key role in the reduction of nickel by *Artemia*.

In the past, research has focused on the use of *Artemia* nauplii as a test organism for a wide variety of contaminants such as metals (Sarabia et al., 1998; Hadjispyrou et al., 2001; Brix et al., 2003), trace elements (Petrucci et al., 1995), pollutants produced by incineration plants (Knulst and Sodergren, 1994), and antifouling compounds (Okamura et al., 2000; Hadjispyrou et al., 2001).

*Artemia* nauplii have also been used in marine toxicity testing in coastal areas (Wells, 1999; Nipper, 2000), and for the establishment of alternative toxicity tests (Calleja et al., 1994; Parra et al., 2001). The above mentioned literature shows the effect of *Artemia* in the toxicity studies, and in the case of the present study *Artemia* similarly acts as a bioaccumulator for the reduction of nickel from the synthetic wastewater.

It was the first time the organism *Artemia* was used in the treatment process. Most of the previous work using *Artemia* was based on toxicity studies and the present work is based on a treatment study. In the present study there are several advantages of using *Artemia*, including their ready availability, ease of culturing, low cost and a large body of literature describing their morphological, biochemical and molecular characteristics (Warner et al., 1989; Macrae et al., 1988), as well as their ability to reduce 87.5% of the Ni. The present investigation has been concerned with the removal of nickel from the bioaccumulation process rather than other biological processes.

**Conclusion**

The present study amply demonstrates the efficient use of *A. franciscana* in the removal of nickel, as this organism appears to be suitable as an environmental bioindicator. The ease of culturing *Artemia* recommends this biological treatment to be an alternative for various microbial treatments. *Artemia* is an effective treatment with many advantages and it could replace the more costly digester systems.

Gradual decrease of nickel was observed from 40 mg/L to 5 mg/L with the removal efficiency of 87.5% with the optimal pH of 9-10 and salinity conditions of 30–35%. MT protein plays a key role in the uptake of nickel by *Artemia*. There was a gradual increase in MT protein observed on 5th day that seemed responsible for the bioaccumulation of nickel in *Artemia* and resulted in 87.5% nickel reduction.

**REFERENCES**


