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Converting of hard water to soft water using fungal bioremediation technology

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ABSTRACT

Objective: To investigate the efficiency of biological agents used for the removal of calcium chloride in water under different parameters.
Methods: The biosorbents prepared from the three fungi used in the present study were tested for their removal capacity of calcium from various concentrations of metal solutions at different pH values and different temperatures.
Results: The present study indicates that out of the three organisms tested *Rhizopus stolonifer* has showed the efficiency of removing calcium from hard water and rendering them to moderate soft water quality at pH of 6 and temperature of 25 °C recorded at the higher concentration of 5 000 μg/mL of ground water and pure solutions.
Conclusions: It can be concluded that the fungal biomass can show significant removal of metal ions than the conventional absorbents.

1. Introduction

Water is a useful resource that is used in many applications like domestic, industrial, agricultural purposes and it also plays an important role in body metabolism and proper functioning of cells. Although, water is abundant in nature occupying about 71% of the earth surface, only 1% is accessible for human consumption[1]. The water content ranges from 60% to 95% for living organisms, and about 60% for humans[2]. To sustain health, humans should drink 1.5–2.5 L of water/person/day[3]. The major source of water for domestic consumption is ground water. Ground water is one of the best sources for public water supplies in many parts of the world[4]. Approximately 30% of all freshwater on earth is stored as ground water[5]. Unless contaminated by man, ground water is generally clean and safe to drink, free of organisms responsible for the spread of waterborne diseases and there is no need for long and expensive pipelines[6]. But often, ground water has excess Ca^{2+} and Mg^{2+}, which make the water hard[7]. The criteria for water quality is based on the content of the above elements, based on which the Indian Council of Medical Research has given the guidelines: soft water or drinking water contains 75 mg/L of Ca^{2+} and 30 mg/L of Mg^{2+}, while hard water contains more than 200 mg/L of Ca^{2+} and more than 100 mg/L of Mg^{2+}[8]. Calcium and magnesium ions typically are present in combination with sulfate, chloride, carbonate, and bicarbonate. These minerals generally are measured in either parts per million or as mg/L. There are some physical and chemical methods which are not that much useful for the conversion of hard water to soft water. The main aim of water treatment technologies is to remove unwanted contaminants from the water and make it useful for drinking and many other applications. Many processes are available for this, and the advantages and disadvantages of these processes have been discussed in reports[1]. So, there is an urgent need to find out an alternative method for removal of metals[9]. One of the alternative method is bioremediation technology. It is defined as a spontaneous process in which biological catalysis acts on

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contaminants and thereby eliminates environmental contamination from the hard water and then the water is portable for domestic use.

The biological agents like fungi have been proved as efficient and economical for the removal of metals from dilute aqueous solutions by biosorption method because the fungal biomass offers the advantage of having a high percentage of cell wall materials, which shows excellent metal binding properties. The biosorption method results not only in metal removal but also provides an eco-friendly environment[10].

2. Materials and methods

2.1. Study area and water samples collection

The study area was Krishna district mainly from Vijayawada urban, Krishna lanka, Machilipatnam, Pamarru, Kankipadu, Gudivada, Avanigadda.

The water samples collected from various bore wells covering the study area. Volumetric and instrumental techniques were adopted for systematic analysis of the water samples using standard procedures. The analysis was carried out immediately for the calcium chloride removal from ground water and pure solutions of higher concentrations and lower concentrations by using three different fungal strains under different parameters.

2.2. Media preparation and maintanance of cultures

Media composition, growth conditions and metal ion estimation procedures for *Aspergillus niger* (*A. niger*, *Neurospora crassa* (*N. crassa*), *Rhizopus stolonifer* (*R. stolonifer*) were described in earlier studies[11-13]. In brief, the test organisms were cultured in basal medium containing glucose (20 g), KH_2PO_4 (3 g), $NH(NO_3)_2$ (2 g), $(NH_4)_2C_4H_4O$ (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), NaCl (0.1 g), $CaCl_2$ (0.1g), trace elements [Zn (200 pg), Mn (200 pg), Cu (80 pg), Fe (20 pg), Mo (20 pg)], biotin (5 pg) in 1 L deionized distilled water.

2.2.1. Preparation of fungal biomass as biosorbent material

A. niger, *N. crassa*, *R. stolonifer* were grown in 250 mL basal medium in 1 L Erlenmeyer flasks and incubated at (28 ± 1) °C under shaking conditions in a rotary shaker at 100 r/min for 3 days. The biomass collected by the above procedure was filtered from the medium using cheese cloth. The harvested biomass was washed thrice with glass-distilled water. Growth of fungal biomass was quantitated by measuring fresh weight of the biomass. The biomass may be stored as suspension in double distilled water containing sodium azide (0.05%).

2.2.2. Preparation of biosorb

The biomass thus obtained was subjected to 5% KOH which degrades the organic material. The inactivated biomass was washed extensively with tap water and again with distilled water to obtain neutrality. The non-living biomass thus obtained without organic matter is referred as biosorb.

2.2.3. Loading capacity of fungi

In order to determine the loading capacity, biomass (1 g) was treated with 12 mol/L HCl for 5 min in order to leach out the surface bound metal ions. Then this acidified biomass was washed thoroughly with distilled water to obtain the neutrality.

2.3. Biosorption experimental method

The present study included three experiements and in each experiment, 50 mL of Erlenmeyer flasks were taken. In each flask, 1 g of fungal biosorbents of the three test organisms *A. niger*, *N. crassa* and *R. stolonifer* were suspended in tap water, drinking water and in 1 000, 3 000 and 5 000 µg/mL of calcium chloride containing pure solutions at 28 °C for 2 h on a rotary shaker at 100 r/min. The biosorption experiment parameters were maintained throughout the experiments. After incubation, the fungal biomasses were harvested and the residual calcium ions were measured using an atomic absorption spectrometer. For each experiment, a blank containing the metal ions solution without biosorbent and a control with distilled water (no metal ion added) and 0.04 g of biosorbent was prepared as well. The values presented in this study were means of three replicates and expressed as standard deviation. The effects of different physical parameters on calcium removal, such as pH, temperature and metal ion concentration were studied. The metal concentrations used were 1 000, 3 000, 5 000 µg/mL, pH values were 4, 5, 6 and 7 and temperatures were 10 °C, 25 °C, 35 °C and 45 °C, respectively.

3. Results

Removal of calcium from ground water and pure solutions of higher and lower concentrations were studied by using the three physical parameters: metal ion concentration, pH and temperature.

The amount of biomass obtained was *A. niger* (110 g), *N. crassa* (60 g) and *R. stolonifer* (90 g), respectively.

3.1. Effect of metal concentration

The metal concentration is an important parameter in biosorption process, which influences the adsorption of metal to the biomass surface. The results presented in this study indicated that calcium biosorption was increased with increasing the calcium concentration at 5 000 μg/mL at 120 min time intervals (Figures 1–3). This is because of the higher concentration of metal ions the amount of adsorbed ions is greater than the amount at a lower concentration since there are more binding sites for interaction.

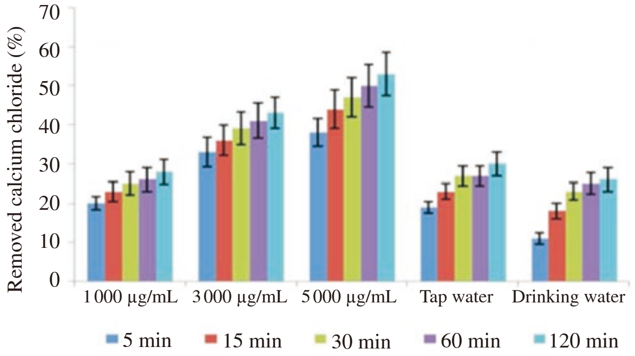

Figure 1. Removal of calcium chloride from ground water and pure solutions of higher concentrations by *A. niger*.

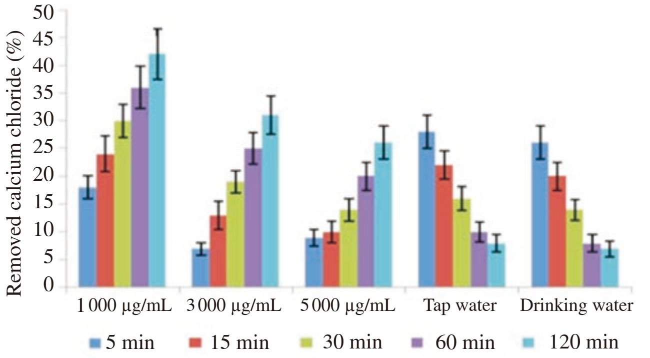

Figure 2. Removal of calcium chloride from ground water and pure solutions of higher concentrations by *N. crassa*.

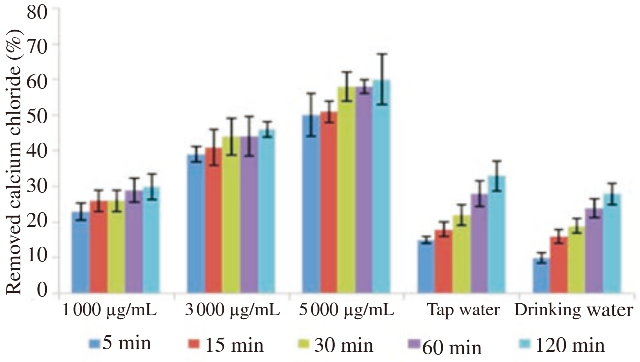

Figure 3. Removal of calcium chloride from ground water and pure solutions of higher concentrations by *R. stolonifer*.

Figure 1 shows that *A. niger* has removed 26% of calcium chloride from drinking water, 30% from tap water, 53% from 5 000 μg/mL, 43% from 3 000 μg/mL, and 28% from 1 000 μg/mL solutions; Figure 2 reveals that *N. crassa* has removed 7% of calcium chloride from drinking water, 8% from tap water, 26% from 5 000 μg/mL, 31% from 3 000 μg/mL, and 42% from 1 000 μg/mL solutions; while Figure 3 shows that *R. stolonifer* has removed 28% of calcium chloride from drinking water, 33% from tap water, 60% from 5 000 μg/mL, 46% from 3 000 μg/mL, and 30% from 1 000 μg/mL solutions.

3.2. Effect of pH

pH is the factor affecting the biosorption behavior of metal ions from aqueous solutions. Calcium chloride removal increases as pH increases in the metal solution up to the maximum pH of 6 and decreases thereafter (Figures 4–6). High efficient percentage of calcium chloride was removed by *R. stolonifer* than *A. niger* and *N. crassa* at the maximum pH of 6. Protons compete effectively with the metal in binding to the functional groups.

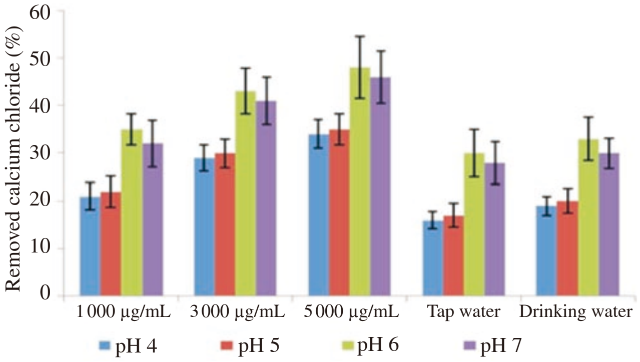

Figure 4. Removal of calcium chloride from ground water and pure solutions of higher concentrations at different pH conditions by *A. niger*.

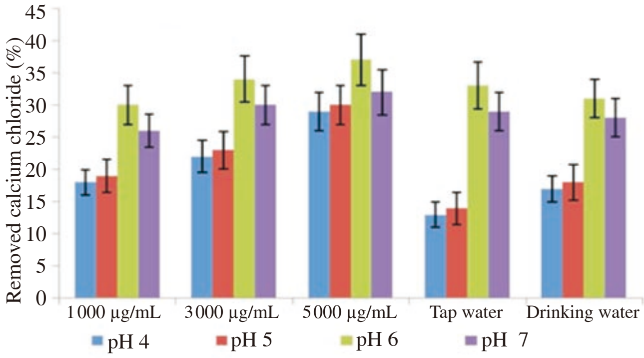

Figure 5. Removal of calcium chloride from ground water and pure solutions of higher concentrations at different pH conditions by *N. crassa*.

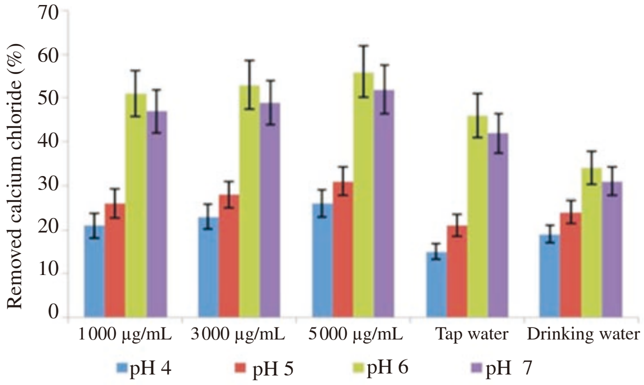

Figure 6. Removal of calcium chloride from ground water and pure solutions of higher concentrations at different pH conditions by *R. stolonifer*.

Figure 4 shows that *A. niger* has removed 33% of calcium chloride from drinking water, 30% from tap water, 48% from 5 000 μg/mL, 43% from 3 000 μg/mL, 35% from 1 000 μg/mL solutions at the maximum pH of 6; Figure 5 shows that *N. crassa* has removed 31% of calcium chloride from drinking water, 33% from tap water, 37% from 5 000 μg/mL, 34% from 3 000 μg/mL, 30% from 1 000 μg/mL solutions at the maximum pH of 6; while Figure 6 reveals that *R. stolonifer* has removed 34% of calcium chloride from drinking water, 46% from tap water, 56% from 5 000 μg/mL, 53% from 3 000 μg/mL, 51% from 1 000 μg/mL solutions at the maximum pH of 6.

3.3. Effect of temperature

Temperatures have a substantial influence on biosorption. Temperature influence has more effect in a situation where by metal uptake increases within a temperature range of about 20–30 °C, but decreases with an increase of temperature above a critical value. An increase of temperature might increase the metal ions kinetic energy which makes it easier for the metal ions to be attached to the biosorbent surface. The result summarized in Figures 7–9 indicated that 25 °C was the optimum temperature at which the higher percentage of calcium chloride was removed by *R. stolonifer*. And also these results indicated that low temperature favoured the removal of calcium chloride by the fungal biomass.

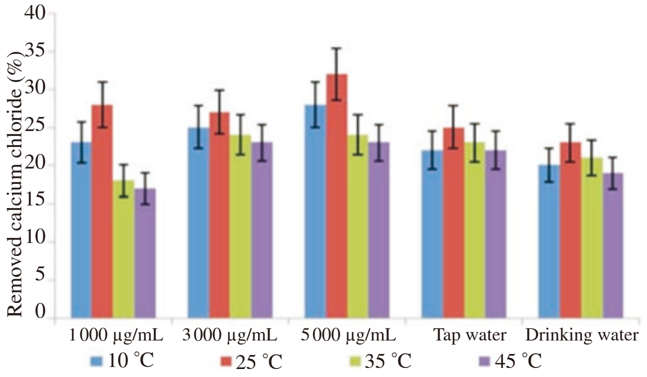

Figure 7. Removal of calcium chloride from ground water and pure solutions of higher concentrations at different temperature conditions by *A. niger.*

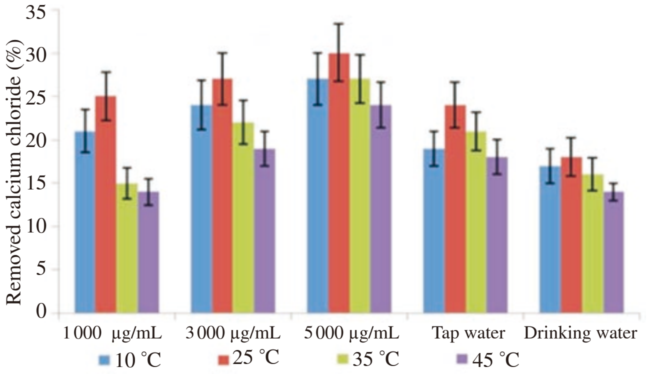

Figure 8. Removal of calcium chloride from ground water and pure solutions of higher concentrations at different temperature conditions by *N. crassa.*

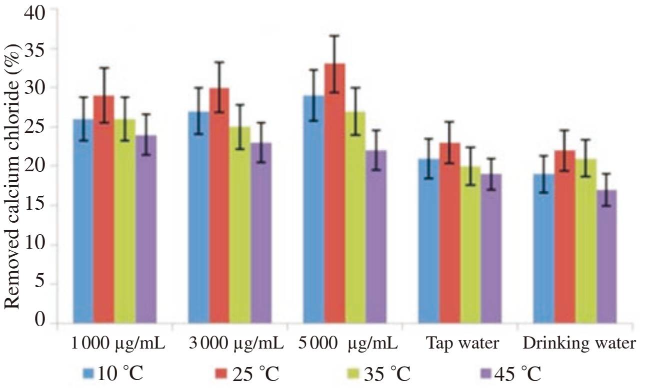

Figure 9. Removal of calcium chloride from ground water and pure solutions of higher concentrations at different temperature conditions by *R. stolonifer.*

It was showed in Figure 7 that *A. niger* has removed 23% of calcium chloride from drinking water, 25% from tap wfater, 32% from 5 000 μg/mL, 27% from 3 000 μg/mL, 28% from 1 000 μg/mL solutions at the maximum temperature of 25 °C; Figure 8 shows that *N. crassa* has removed 18% of calcium chloride from drinking water, 24% from tap water, 30% from 5 000 μg/mL, 27% from 3 000 μg/mL, 25% from 1 000 μg/mL solutions at the maximum temperature of 25 °C; while Figure 9 demonstrates that *R. stolonifer* has removed 22% of calcium chloride from drinking water, 23% from tap water, 33% from 5 000 μg/mL, 30% from 3 000 μg/mL, 29% from 1 000 μg/mL solutions at the temperature of 25 °C.

4. Discussion

Inactivated microbial biomass presents a very useful source for the biosorption of toxic metals. Sufficient quantities of biomass can be obtained as waste product of fermentation industries employing bacteria, yeast and fungi. Due to low mechanical strength, low density and small particle size live biomass as such is not suitable for use in the detoxification of certain effluents. Susceptibility to biodegradation reduces the storage and shelf life of the biomass. Therefore, it is necessary to convert the biomass into a form that is suitable for various designs of bioremediation technology.

There is a dramatic increase in dependence on ground water, which is the prime source for drinking in many parts of the world. But often, ground water has an excess of Ca and Mg, which makes the water hard. Calcium and magnesium ions typically are present in combination with sulfate, chloride, carbonate, and bicarbonate. These minerals generally are measured in either parts per million or as mg/L. The criterion for water quality is based on the content of the above elements, based on which the Indian Council of Medical Research has given the following guidelines. Soft water or drinking water contains 75 mg/L of calcium and 30 mg/L of magnesium. Hard water contains > 200 mg/L of Ca and > 100 mg/L of Mg. Hard water contributes to inefficient and costly operation of water-using appliances. Heated hard water forms limescale deposits that contribute to inefficient operation or failure of water-using appliances. Many physical and chemical methods are employed to remove hardness of the water. Mechanical water softening and ion exchange softeners are generally supersaturated with sodium. According to the Water Quality Association, sodium gets added to about 8 mg/L for each grain of hardness removed. However, no biological method of removing water hardness has been developed till date. The mechanism of binding of toxic metal ions by fungal biosorbents was earlier shown to be by ion exchange, in which the resident calcium is displaced by the toxic metal ions. There is a greater driving force at higher concentration between the solid and liquid interface thus enabling mass transfer[14]. With an increase in pH, the negative charged density on the cell surfaces increases due to deprotonation of the metal binding sites and thus increases biosorption[15]. At high temperature the decrease in the removal of calcium chloride is due to the decrease of either higher affinity of binding sites on relevant cell mass[16].

Regeneration procedures, which results in loss of calcium, were shown to decrease the metal binding capacity of the biosorbent. However, regeneration with calcium containing solutions resulted in regaining metal binding capacity[17,18]. The above consequences indicated that calcium could be rebound by the biosorbent. In the present study, fungal biosorbents were demonstrated to have good potential to remove calcium from hard water. Optimal conditions for removing calcium from hard water were established. Till date this is the first report on using fungal biosorbents for removal of calcium and further studies are needed to see if this approach has any commercial application.

In this study, the adsorption of metal ions of calcium chloride on fungal biomass was successfully achieved. Out of the three test organisms, *R. stolonifer* has showed the efficiency of removing calcium from hard water and rendering it to moderate soft water quality at pH of 6, temperature of 25 °C recorded at the higher concentration of 5 000 µg/mL of ground water and pure solutions. It can be concluded that the fungal biomass can show significant removal of metal ions than the conventional absorbents.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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