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Environmental Study For The Role Of Some Bacterial Species In Wastewater Treatment

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Abstract
S. aureus, bacillus sp, P. putida, P. aeruginosa, and Micrococcus luteus are among the bacterial isolates that will be examined in this research for their potential to lower the levels of certain pollutants in wastewater collected from the final settling ponds regarding the central wastewater treatment plant (Almamierh) in Hilla city. According to the findings, pH values found in this work fell between 6.8 to 7.6, which is within typical limits. A few species recorded somewhat lower oxygen concentrations, but overall, the values have been similar to those in the control. While all of the bacterial species in the presented research performed exceptionally well in reducing the alkalinity values, some of them did better than the control in terms of electrical conductivity as well as dissolved solids removal. In comparison with control, several bacterial species showed a slight effectiveness in removing chloride and a better removal rate of magnesium and total hardness, yet were ineffective at lowering calcium values. Bacillus sp. had the highest rate of nitrate removal, while phosphate had a slightly lower removal percentage.

INTRODUCTION

According to Abatenh et al. (2017), bioremediation is the process by which biologically organic wastes are degraded under controlled settings to safe levels or to concentrations below those previously set by regulatory authorities. Numerous elements, including the environment's chemical and physical characteristics, the concentration and chemical nature regarding pollutants, and their accessibility to microorganisms, all affected how effective bioremediation was (Agathos and El Fantroussi, 2005). When compared to alternative treatment approaches, this technology has a number of advantages, including lesser environmental damage from pollutants and relatively lower prices (Mingjun et al., 2009). It should not include any hazardous chemicals (Sharma, 2012), environmentally beneficial and sustainable (Dell Anno et al., 2012).

Along with being easier to maintain, bioremediation technology is more cost-effective than other chemical and physical treatment methods when it comes to capital investment expenses (Mittal, 2011). Through biodegradation, this application uses microorganisms to eliminate and mitigate the risks associated with several pollutants. Through their enzymatic pathways, microorganisms function as biocatalysts and speed up the biochemical reactions that degrade the target pollutant. Microorganisms are crucial in the destruction of pollutants through bioremediation because they have the enzymes that enable them to use pollutants as a food source in the case when various materials and compounds are available for obtaining nutrients needed for building more cells and generating energy. This allows for the optimal levels of nutrients required for metabolism, which in turn helps to decompose and detoxify substances posing a threat to the environment. All metabolic reactions are carried out by enzymes, which belong to the groups of oxidases, lyase, hydrolase, isomerase, transferase, and ligand. Various enzymes have a broad decomposition capacity, and for treatment to be effective, such microorganisms should enzymatically attack pollutants and transform them into other harmless components (Abatenh et al., 2017; Naji, et al. 2024)).

Since bioremediation generally works only in environments that are conducive to microbial growth and activity, its use frequently entails adjusting environmental parameters to enhance the rate of growth as well as degradation regarding such organisms (Kumar, 2011; Mohi AL-kahfaji, et al. 2023).).

MATERIALS AND METHODS

Bacterial Isolates

This work employed bacterial isolates. P. putida, P. aeruginosa, Bacillus sp., S. aureus, and Micrococcus luteus have been supplied by the Department of Biology's advanced industrial microbiology section at the University of Babylon's College of Science.

Each of the bacterial isolates was inoculated separately in brain-heart infusion broth and incubated for 24 hours at 37°C to activate it. At 600 nm, 1.5 x 108CFU/100 ml of sterile saline solution (0.85%) has been used to bring the O.D. of c0ell suspension down to 0.5. Each isolate's (10%) inoculum has been taken separately in a 250 ml flask with 90 n-sterile wastewater rates. Additionally, a single control flask with solely non-sterile wastewater was employed. For ten days, such flasks have been incubated at a temperature of 37 Celsius at 120 rpm in a shaker incubator. Samples were taken and examined at 0-, 5-, and 10-days following treatment. Every experiment was run in duplicate.

Chemical and Physical Parameters

The pH meter (multi-parameters) has been used to test the pH, EC (µs/cm), and TDS (mg/l) in wastewater. Total alkalinity as well as dissolved oxygen have been measured in accordance with APHA (2003). The Lind (1979) approach was used to quantify total hardness, magnesium, and calcium. The method outlined through APHA (2005) was used for determining the chloride ion. In accordance with Parson et al. (1984), nitrate has been estimated. Riley and Murphy (1962) and Smith (2004) both provided estimates for phosphate.

RESULTS AND DISCUSSION

The concentration of hydrogen ions, or PH, is subject to significant changes that control the metabolism, activity, and species regarding microorganisms that can survive and adapt to a specific pH value and die when that value changes. Since pH influences the availability of nutrients and minerals, it is one of the most significant factors influencing the physiology as well as metabolism of aquatic organisms. It is one of the elements that influences the existence of bacteria in water and is the outcome of a series of biological and chemical reactions (Lawson, 2011; Jassim, *et al.* 2023)). Numerous ions, including bicarbonates and carbonates, as well as dissolved gases, including CO2 and hydrogen sulfide, affect hydrogen concentrations. The present work's measured values fell within normal limits, ranging from 6.8 to 7.6. They have a tendency to be slightly alkaline, and the water treated with P. putida showed a very minor increase on days 5 and 10. On the other hand, the water treated with S. aureus showed the lowest values on day 10 (Figure 1). The pH variation in wastewater indicates the presence of microorganisms that degrade organic substances (Garode and Sonune, 2015). The amount of carbon dioxide, the activity of microorganisms, and the processes of photosynthesis and respiration are just a few of the variables that affect pH adjustment (Trimborn, 2008). Since several compounds, including mineral acids, are formed during the bio-degradation as well as oxidation processes, the reduction might be connected to the decomposition regarding wastes. All aquatic organisms' metabolic functions depend on dissolved oxygen, which is either provided to the water by photosynthesis or the atmosphere (Linkens and Wetzel, 2000). Numerous variables, including salinity, temperature, oxygen partial pressure, organic compounds, and the density of aquatic plants as well as phytoplankton, affect how soluble oxygen is (Ahangar et al. 2012). A few species reported somewhat lower amounts than the control sample, according to the present work's results, and the oxygen values have been comparable to those found in the control sample (Figure 2). A rise in the decomposition of organic matter by microorganisms might be the cause of the lack of an increase in oxygen values (Hassan, 2004). Bacteria could produce a wide range of enzymes that could degrade complex compounds into CO2 and water and use the compound as a fuel for cellular respiration and protein production.

As shown in Figure (3) (A, B), S. aureus and P. putida each recorded the highest reduction rates (42.8% and 26.12%) on days 10 and 5, respectively, indicating that while certain bacterial species in the presented work did not demonstrate efficiency in decreasing dissolved solids values, other species documented efficiency in removal when put to comparison with the control. Since TDS is composed of both negative and positive ions, including sodium salts, calcium and magnesium salts, chlorides, bicarbonate, sulphate, and dissolved organic matter, the observed removal rates might be the result of certain bacterial species' effectiveness in removing certain ions from the medium where they grow (Duffy and Weber, 2007). The capability of water to transport an electric current is known as electrical conductivity. Because of its tight relation to total solids, it serves as an indication of dissolved salts in water (APHA, 1976). The efficiency regarding the bacteria under investigation was straightforward in lowering electrical conductivity values, as demonstrated by Figure (4) (A, B). The maximum decrease percentage was achieved by the types P. putida and P. aeruginosa at days 10 and 5, respectively, at 12.2 and 11.08. The excessive concentrations of salts that are above the bacteria's capacity to eliminate them could be the cause of the bacteria's poor efficacy in lowering conductivity values. When cations like potassium, sodium, calcium, and magnesium are present, as well as anions, like bicarbonates, carbonates, chlorides, nitrates, and sulfates, electrical conductivity values rise (Maiti, 2004).

In neutral conditions, the alkalinity—a measurement of weak acids and their salts—represents an integrated and balanced system of CO2-bicarbonate-carbonate (Hussain et al., 1991). Additionally, it has a significant impact on pH regulation (Weiner, 2000). According to Lind (1979), it is a reliable indicator of the water content of hydroxides, carbonates, bicarbonates, and other ions. On the fifth day, all bacterial species outperformed the control in terms of eliminating total alkalinity, with P. aeruginosa achieving the highest removal percentage (44.6%) (Figure 5) (A, B). Bacteria might be effective at lowering total alkalinity values because they produce extracellular polymeric materials (EPS), which are crucial for microbial calcification (Vickers, 2017). EPS might trap a lot of divalent cations, like Mg2+ and Ca2+, because it contains a variety of acidic residues and sugars (Kremer et al., 2008). Additionally, EPS contains metallic binders, including phosphate, carboxyl, amine, and hydroxyl groups, which bind to those negatively charged groups for removing free cations from solution (Tourney et al., 2008).

According to Gupta et al. (2009), hardness is a reliable indicator of the presence of common divalent ions like magnesium and calcium as well as the involvement of additional ions, such as iron, barium, stronium, manganese, and zinc. On the tenth day, certain bacterial species showed a somewhat greater removal rate compared to the control; Bacillus sp. had the highest percentage of removal (31.2) in Figure (6) (A, B). The study's bacteria have been ineffective at lowering calcium values, as seen in Figure (7) (A, B). In contrast to the control, several bacterial species shown effectiveness in eliminating magnesium; at days (5) and 10, respectively, the greatest removal rates for P. putida and Bacillus sp. were 52.8 and 62.5 (Figure 8) (A, B). Microbial cell surfaces are negatively charged and function as scavengers for cations in aquatic environments by attaching them to their cell surfaces, which might account for the effectiveness regarding bacteria in removal (Ramachandran et al. 2001).

Natural water contains the negative ion chloride. Particularly when it attaches to the sodium ion to produce sodium chloride, the water takes on a salty flavor. On the fifth day of treatment, certain bacterial species did not demonstrate any effectiveness in removing chloride, whereas other species shown a slight efficiency in the removal process when put to comparison with the control. P. aeruginosa had the highest removal rate (16.4%) (Figure (9) (A, B). Since chloride ion makes up the extracellular fluid of microorganisms, its partial degradation or removal might be attributed to its consumption through such bacterial species. The content of chloride ions has decreased since they are a major component of extracellular fluid and are used for photosynthesis as well as bacterial growth (Awadallah et al., 1998; shaemaa , et al, 2024). The chloride ion, which is present in polluted and waste water at high concentrations as NaCl, might make it more difficult for bacterial species to eliminate it, which could explain why they are ineffective at lowering the concentration of chloride.

The biological removal of inorganic nitrogen in wastewater treatment plants involves a variety of processes, including nitrification, ammonification, denitrification, and anammox processes. Inorganic nitrogen is typically available in a variety of forms, including nitrite, ammonia, and nitrate (Guo et al., 2020; Liu et al., 2020). According to Rahimi et al. (2020), biological nitrogen removal involves activities that change nitrogen compounds into a variety of oxidation states. The roles of various microorganisms as well as metabolic reactions vary in each one of the processes, and each nitrogen removal method has a variable level of efficiency (Zhang et al., 2021b). In comparison to the control, the bacterial species under investigation in this study removed the most nitrate, with Bacillus sp. recording the highest removal rates (77.03 and 57.4) at days (5) (10), respectively, Figure (10) (A,B). As an essential nutrient as wlel as electron acceptor in microorganisms, nitrate is one of the key nitrogen components in the environment, which might explain the observed removal values. According to Galloway et al. (2008), nitrate input could affect N-cycling activities, which in turn might support the biogeochemical processes that are nearly entirely mediated through microorganisms. The highest nitrate removal rate (75%) has been found in pond water treated with Bacillus sp. in research conducted via Thurlow et al. (2019). Following Bacillus sp. treatment, Lalloo et al. (2007) observed a comparable decrease in nitrate ions in synthetic pond water. Additionally, Zokaeifar et al. (2014) and Hura et al. (2018) both reported the highest nitrate removal rates.

Active Phosphates (Orthophosphates) are soluble in organic form and are utilized via living organisms. Phosphates are an intermediary component in the processes regarding energy metabolism for all living organisms. In comparison with the control, which did not record any removal rates, the two species, M. luteus and S. aureus, recorded low removal rates at day (5) of treatment, yet greater removal rates from the bacteria under study at day (10) of treatment, as shown in Figure (11) (A, B). High concentration gradients of various ions might cause ions to leak across the cell membrane, impairing the ability of some bacterial species to reduce phosphate values. Once inside the cell, the ions must be expelled from intracellular environment, which could disrupt metabolic processes in various ways. As a result, the cells must export the ions against the concentration gradient at the expense of energy, potentially resulting in cycles that are pointless (Castle et al., 1986). Additionally, in the case when biological processes are required for treating saline wastewaters, the salinity might impact the microorganisms that are in charge of various nutrient removal processes. In the case when microorganisms are exposed to salinity, they must contend with a number of chemical and physical stressors, including osmotic pressure, ionic strength (Truper and Galinski, 1994; Muller and Roesler, 2001), and significant concentration gradients of various ion types (Castle et al., 1986).

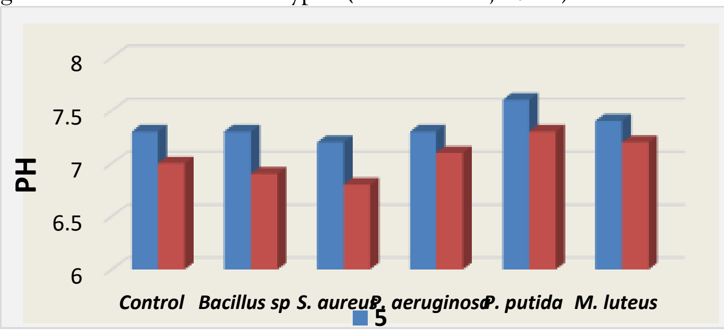


Figure 1: concentrations of PH

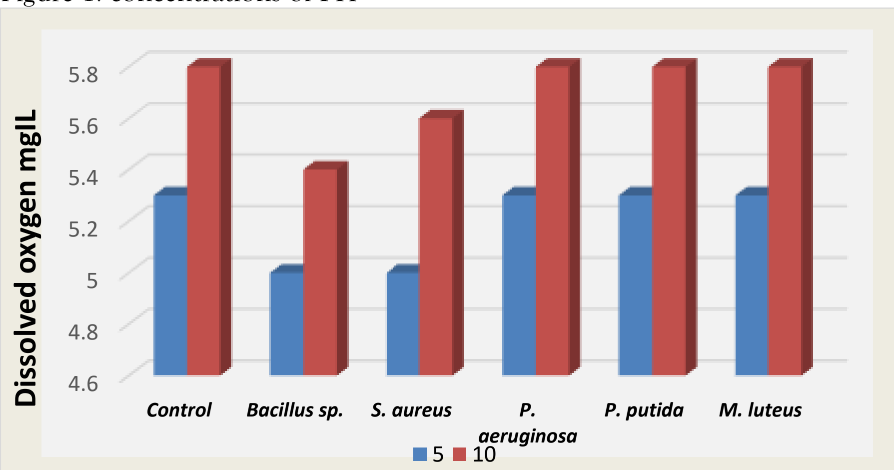
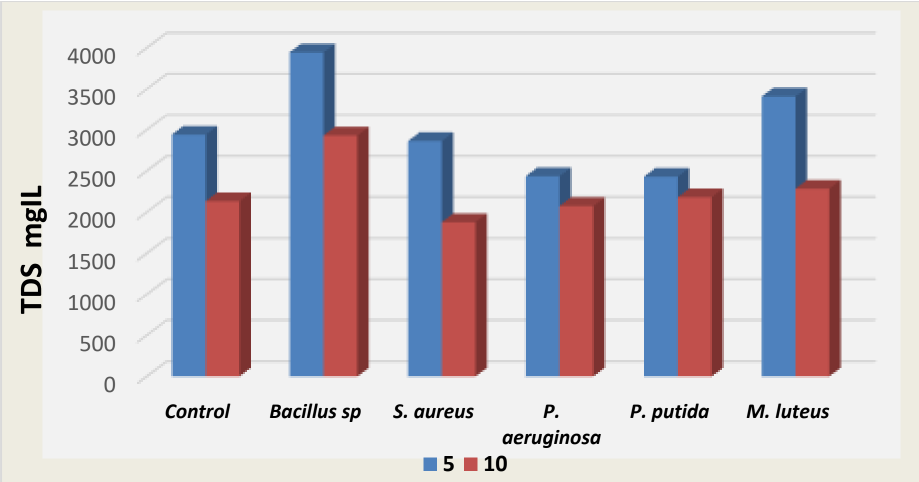


Figure 2: concentrations of Oxygen



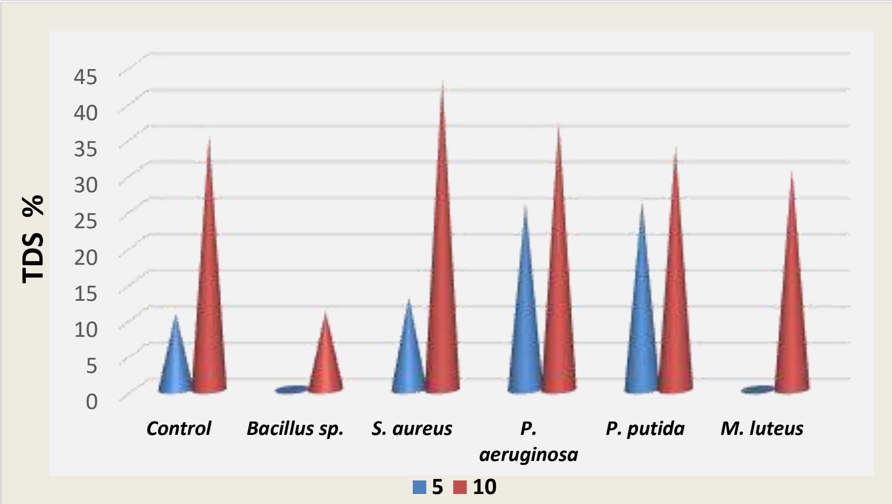
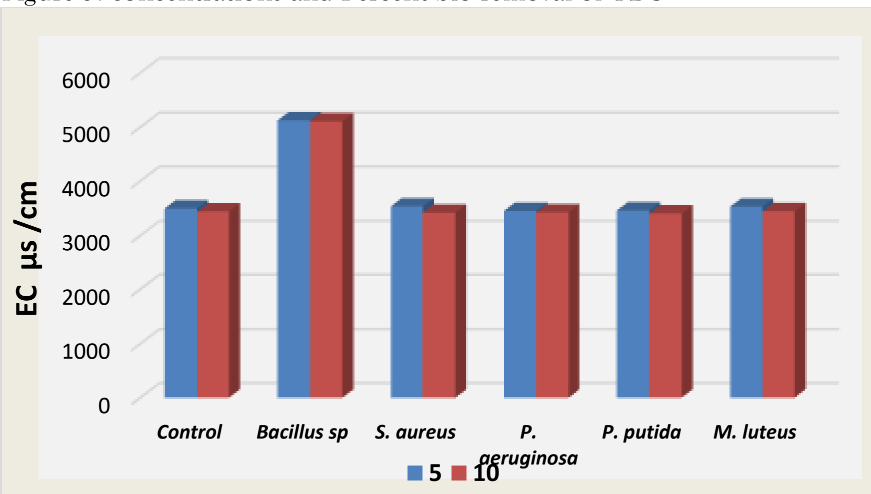


Figure 3: concentrations and Percent bio removal of TDS



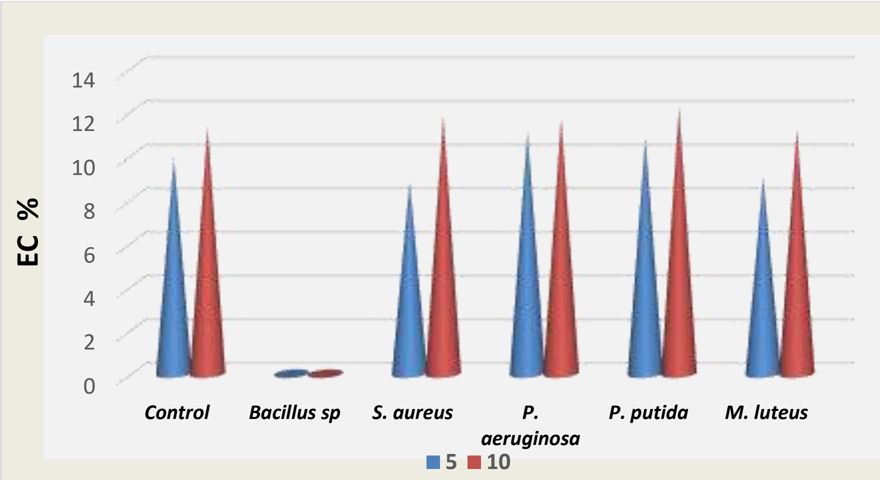


Figure 4: concentrations and Percent bio removal of electrical conductivity

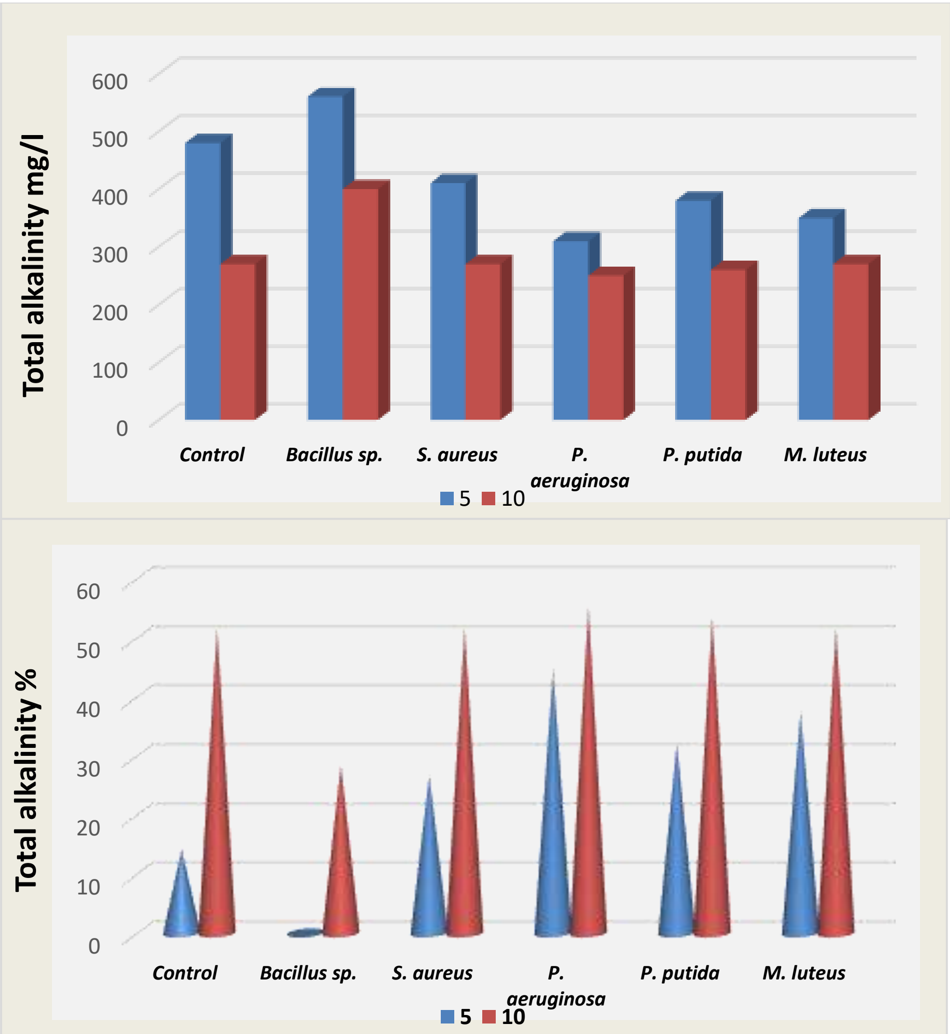
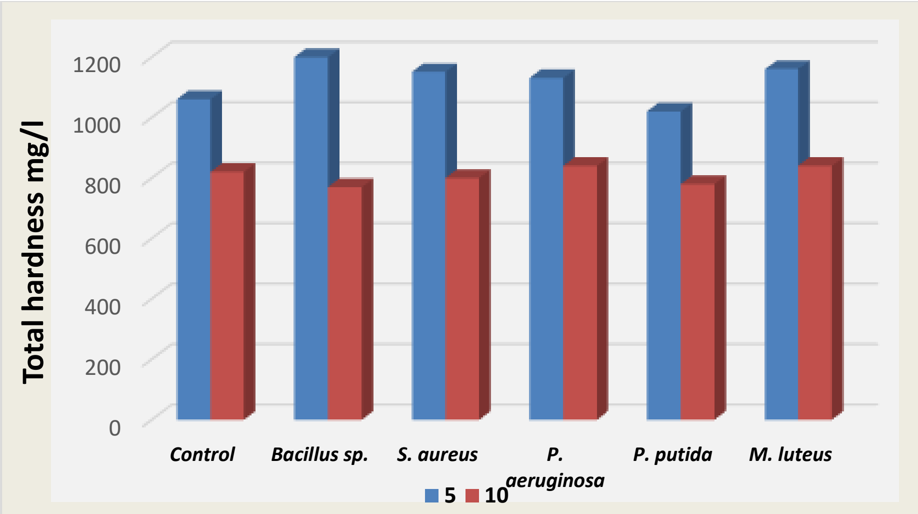


Figure 5: concentrations and Percent bio removal of Total alkalinity



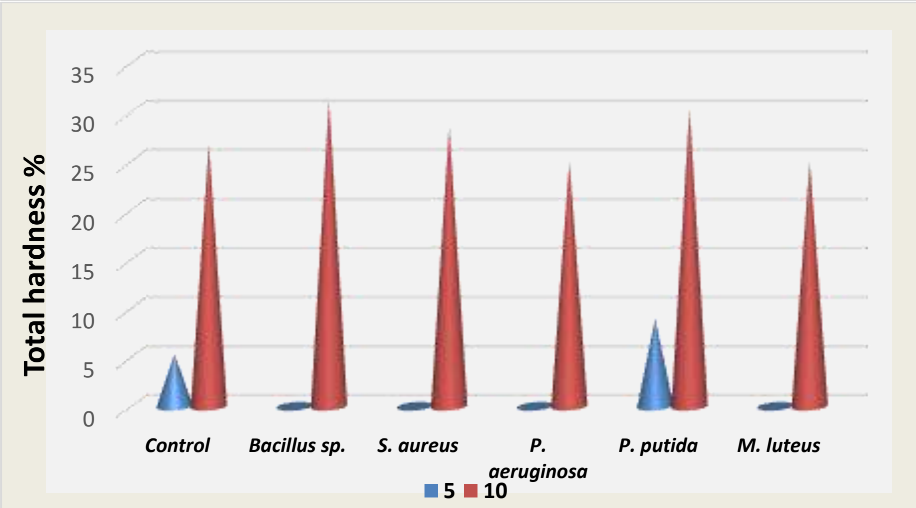
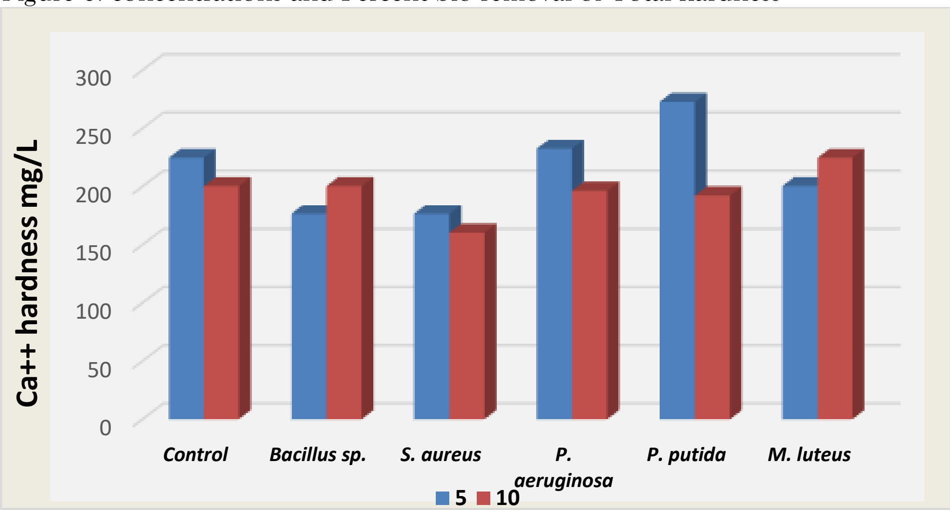


Figure 6: concentrations and Percent bio removal of Total hardness



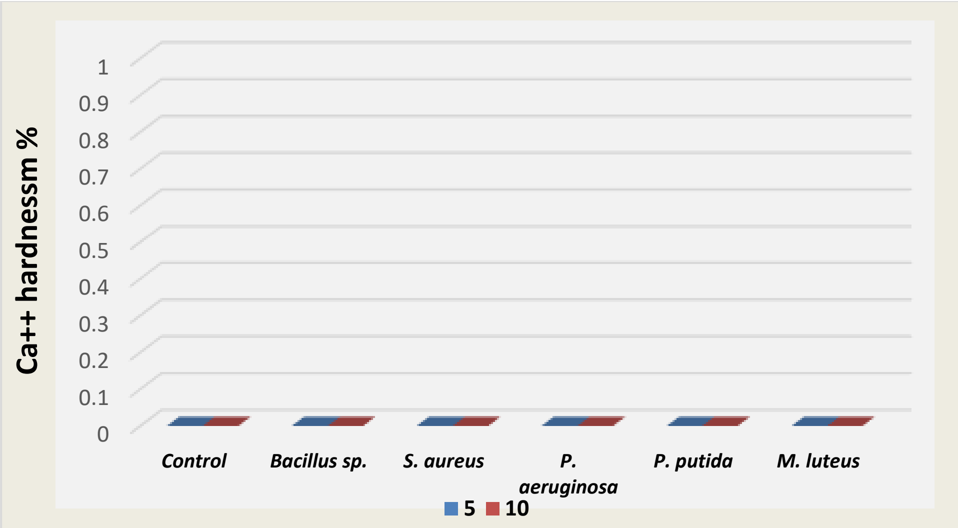


Figure 7: concentrations and Percent bio removal of Calcium (Ca)

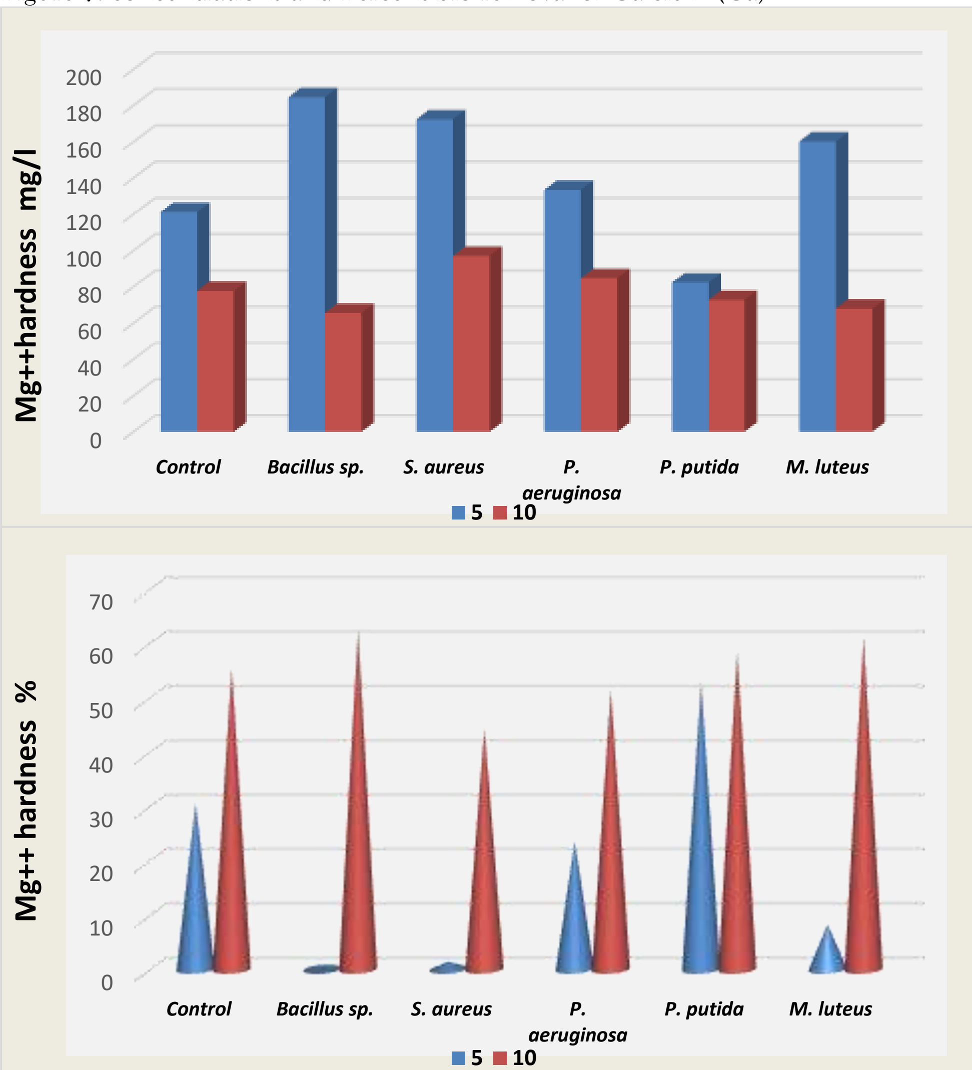
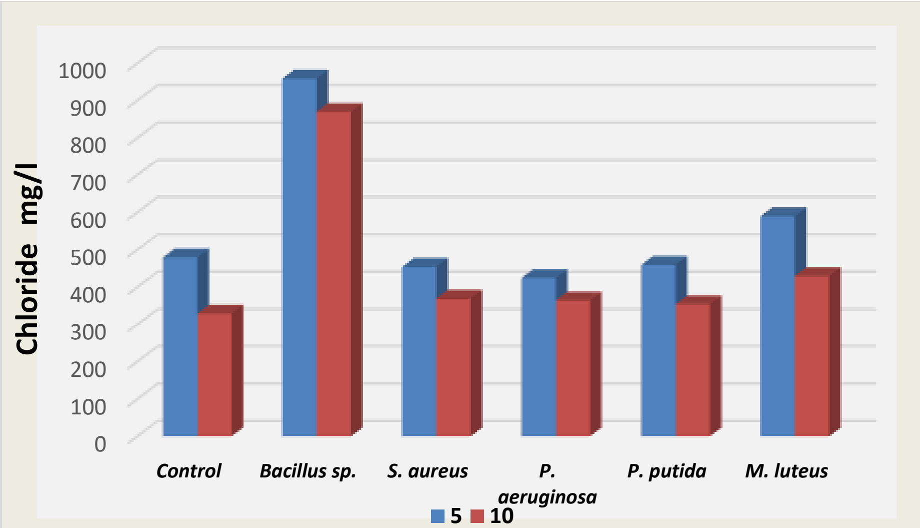


Figure 8: concentrations and Percent bio removal of magnesium(Mg)



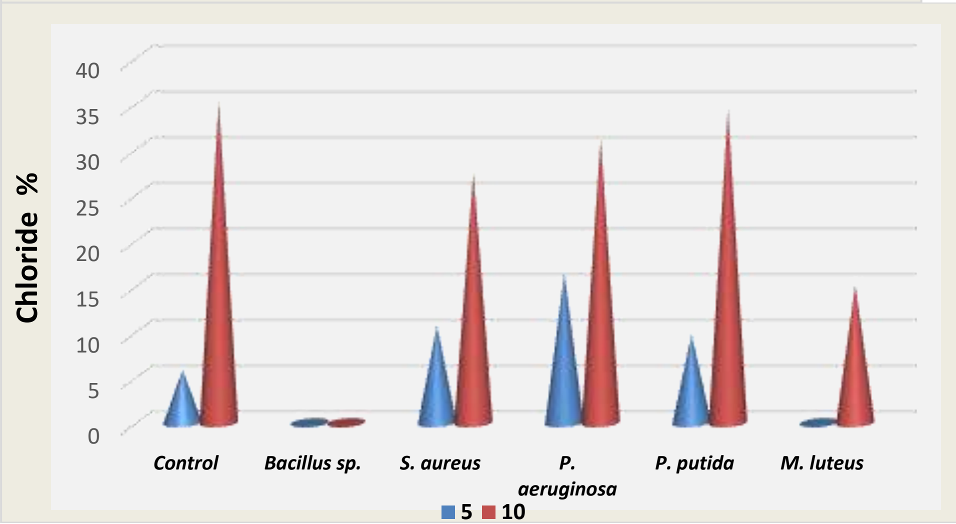
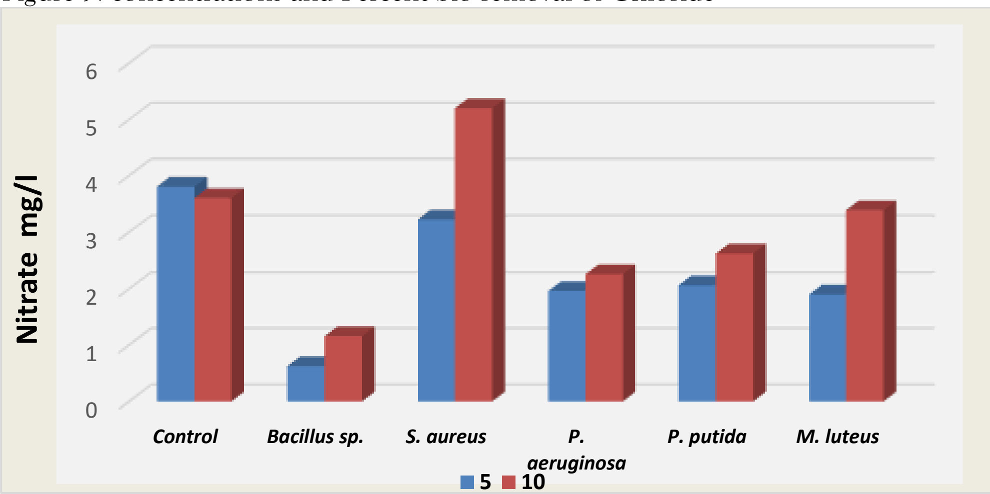


Figure 9: concentrations and Percent bio removal of Chloride



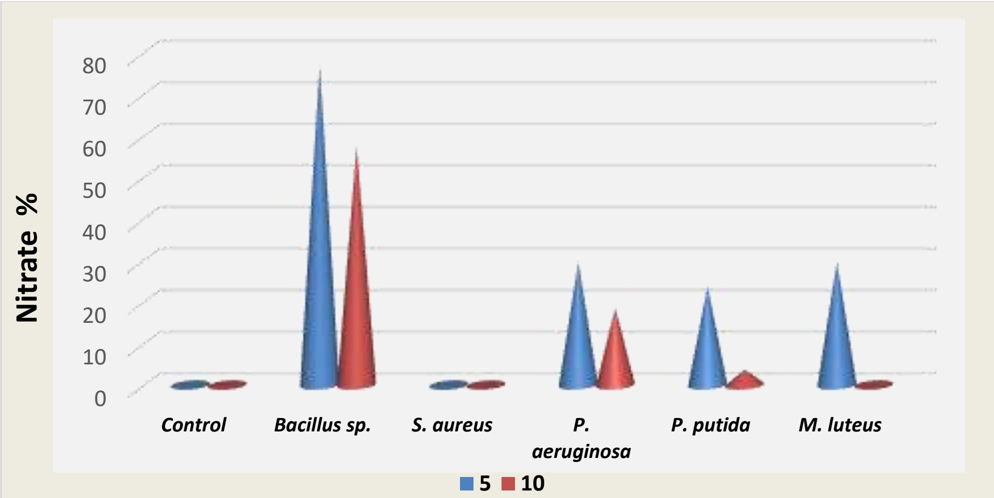


Figure 10: concentrations and Percent bio removal of Nitrate

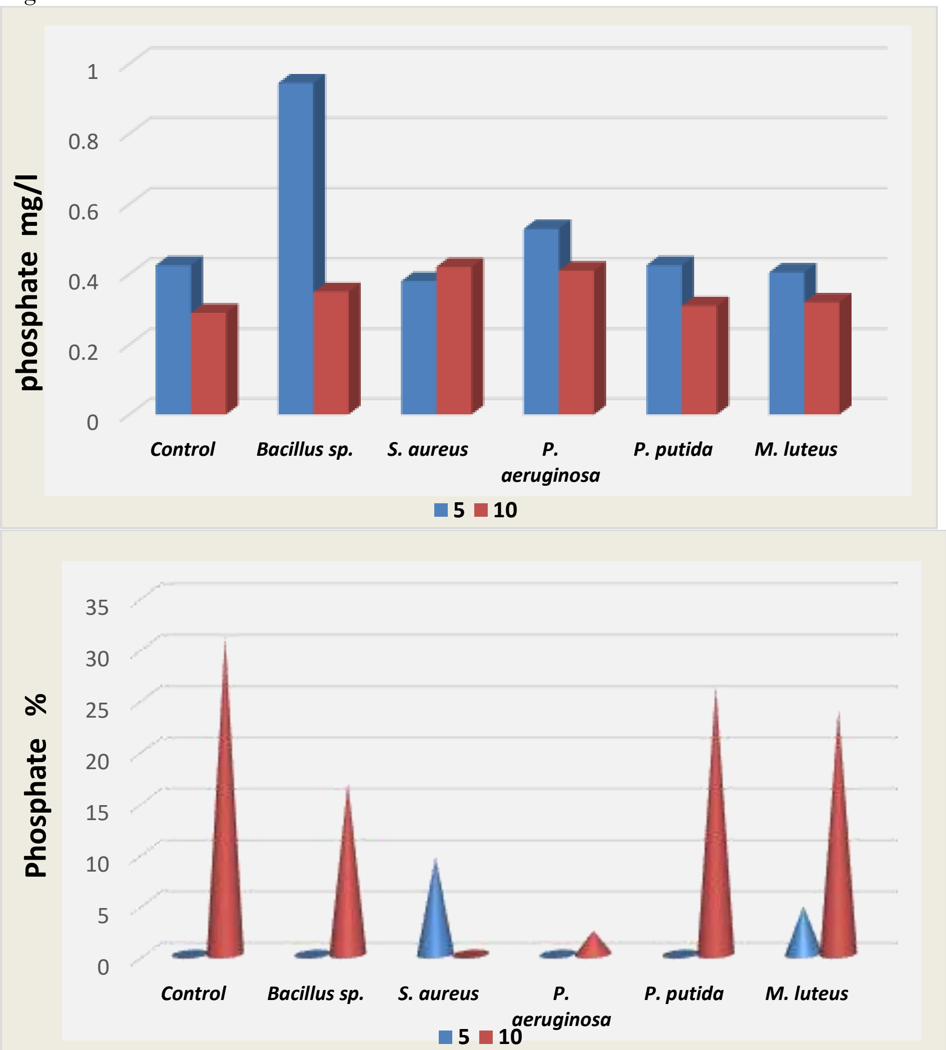


Figure 11: concentrations and Percent bio removal of phosphate

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