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Bioremediation of Nitrate in Pharmaceutical Waste

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ABSTRACT
Pharmaceutical industries are commonly employed to manufacture a wide range of antibiotics, solvents, and other by-products that are commonly used by humans. The waste materials are released in streams, soil, and air, it endangers human life and has a negative impact on the environment.Biodegradation of nitrate from pharmaceutical waste is essential. Many bacterial strains are able to biodegrade nitrate from explosive industry effluent. Pseudomonas speciesable removingthe nitrate and nitrite from the nitroglycerine manufacturing pharmaceutical waste effluent. The goal of research is to use bioremediation to remediate industrial effluents used in creation of by-products in medication manufacturing to reducenitrate.
Keywords: Bioremediation, denitrification

I. INTRODUCTION

Pharmaceutical industry are geographically dispersed, and medication development has resulted in a constant global growth in the usage of powerful pharmaceuticals. Varity of pharmaceuticals can now be detected in ground, surface, and drinking water, there are legitimateworries about the potential for environment impact as a result of contamination. Nitrate is one of the major inorganicpollutant produce bypharmaceuticals industries. This has aggravated acidification of soil and water bodies, altered ecosystem species composition, increased nitrate levels in drinking water beyond safe levels, and caused eutrophication of lakes and the sea.Nitrate is prone to leaching to the subsurface layers and eventually to the groundwater because of its high solubility in water and low retention by soil particles. When ground water with high nitrate concentration is used for drinking,it poses a risk to public health, especially to newborns.

Methemoglobinemia, goiter, stomach cancer, and deformed children are all health risks connected with excessive nitrate levels. Infants are more vulnerable to methemoglobinemia than adults because their stomach acidity is lower, allowing bacteria capable of converting nitrate to nitrite to proliferate. Drinking water containing the high amount of nitrates can result in the birth of a kid who is deformed. Other health problems have been reported, including non-Hodgkin lymphoma, increased newborn mortality, and hypertension. As result, nitrate-contaminated wastewater must be treated effectively before being discharged onto the land or into stream. Despite the fact that various chemical treatments exist, they are ineffective in removing nitrate. It is also cost-effective and cause disposal issues due to the leftover solid waste.

Many studies have focused on biological removal of nitrogenous compound from industrial waste water with high nitrogen content, primarily due to its potential advantage and enhanced removal of these compounds over physical and chemical procedure. biological treatment contains both anaerobic and aerobic systems in which nitrate is digested by microorganisms in three ways: aerobic assimilation to ammonia, aerobic or anaerobic dissimilation to nitrogen, and anaerobic assimilation to ammonia.

High nitrate levels endanger human and animal health by accumulating nitrate or converting it. As a result, the goal of this study was to see how effective microorganisms are at removing nitrate from waste streams from explosive production factories, both individually and in groups.

II. MATERIALS AND METHODS

The pharmaceutical industry effluent was obtained from drug manufacturing plant located in Gujarat,India that produce Nitroglycerine. The effluents were analyzed for following physiochemical parameters: pH, sodium, potassium, phosphate, dissolved oxygen, nitrate, nitrite, and ammonia.

Isolation and Identification of pseudomonas aeruginosa:

One gram of collected soil sample was weighted and added in sterile test tube containing 9ml of distilled water to make serial dilution of 10^{-}

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^{2}to10^{-6}using a serialpipette each time. Sterilized 100ml nutrient agar medium prepared by autoclaving for 15 min at 121°C.when media was cooled poured it into three sterilized petri-dishes. Allow the plates for solidify. spread 0.2 ml serial dilution of soil sample from 10^{-2},10-4 and10^{-6}on petri-dishes. Incubate all three plates at 37°Cfor 24 hr.

Physiological and biochemical testing were performed on the isolates. The isolates were identified by various biochemical tests such as oxidase test, catalase test and using specialized media.

Removal of nitrate by pseudomonas aeruginosa:

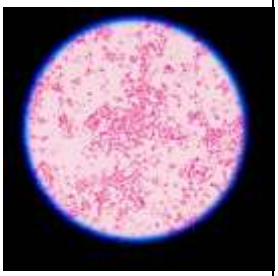
Prepared three sterilized test tubes labeled as control, positive and negative. Nitroglycerine effluent were present inside positive and negative labeled test tube and nitrate broth [peptic digest of animal tissue 5.00 gm/liter, beef extract 3.00 gm/liter, potassium nitrate 1.00gm/liter, pH 7.0] was in control. Addition of nutrient broth [beef extract 1.00 gm/liter, yeast extract 2.00 gm/liter, peptone 5.00 gm/liter, sodium chloride 5.00 gm/liter, pH 7.4] inside two test tube containing nitroglycerine effluent. Control and positive labeled test tubes were inoculated with pseudomonas aeruginosa and incubated all three tubes were incubated at 37°C. After the result observation, measurethe effect of pseudomonas aeruginosa on nitroglycerine effluent by using different time period 24 hour, 48 hour and 72 hours. Nitrate concentration were monitored by using UV spectrophotometer at 410 nm.

Nitrite detection is a two step procedure that involves the addition of nitrate reagent A (sulfanilic acid) followed by reagent B(alpha-naphthylamine) to determine the reduction of nitrate to nitrite. The presence of nitrite is confirmed if red tint appears. If no color change occurs, either nitrate or nitrite remain unreduced. This is determined by adding zinc powder, a strong reducer that converts nitrate to nitrite, turning the culture red and confirming the presence of unreduced nitrate in the tube. If no color change after adding zinc powder, the only reason is that nitrate was converted to nitrite, which was subsequently converted to other nitrogen molecules, such as ammonia.

III. RESULT AND DISCUSSION

The isolated bacterial strain gave the white color colonies on nutrient agar media, morphologically gram-negative, rod shaped, biologically gave positive oxidase and catalase test and gave the fluorescence green colonies on specialized king's agar media. It confirmed that isolated bacterial strain is pseudomonas aeruginosa.

Gram reaction and motility test:

Name of medium	Gram staining	observation	Motility
Nutrient Agar plate	**Size: small Shape: rod Arrangement: single Color of organisms: pink Color of background: colorlessMicroscopy: bright field Gram reaction: gram negative**		Motile organisms

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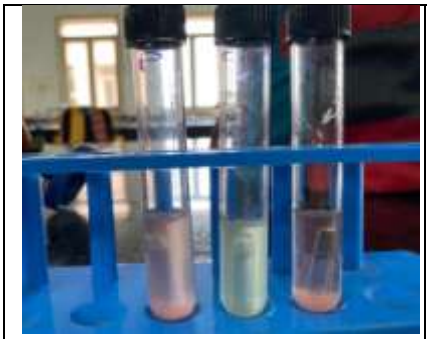
Result of Biochemical test:

Test	Observation	Result
Oxidase test		**positive**
Catalasetest		**positive**
Simmonscitrate		**positive**

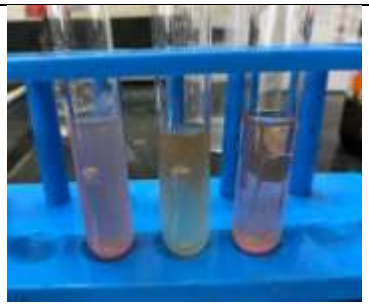
Growth characteristics on king's agar plate:

Media	Observation
King's agar plate	 **Flurocent green color colonies**

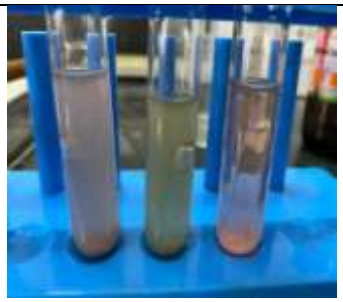
Nitrate reducer:



Presence of the gas inside positive and control tubes



No color changes after addition of reagent A (sulfanilic acid) and reagent B (alpha-naphthylamine)



No color changed after addition of zinc

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There was no changes in color after addition of nitrate reagent A (sulfanilic acid) and reagent B (alpha-naphthylamine) further addition of zinc also couldn't change the color of medium. There was also the presence of gas inside the Durham tube and media was able to convert red litmus paper to blue. Because isolated bacterial strain may be able to produce nitrate reductase and nitrite reductase which converts the nitrate inside the nitroglycerine into nitrite and further converts in nitrogen gas or ammonia.

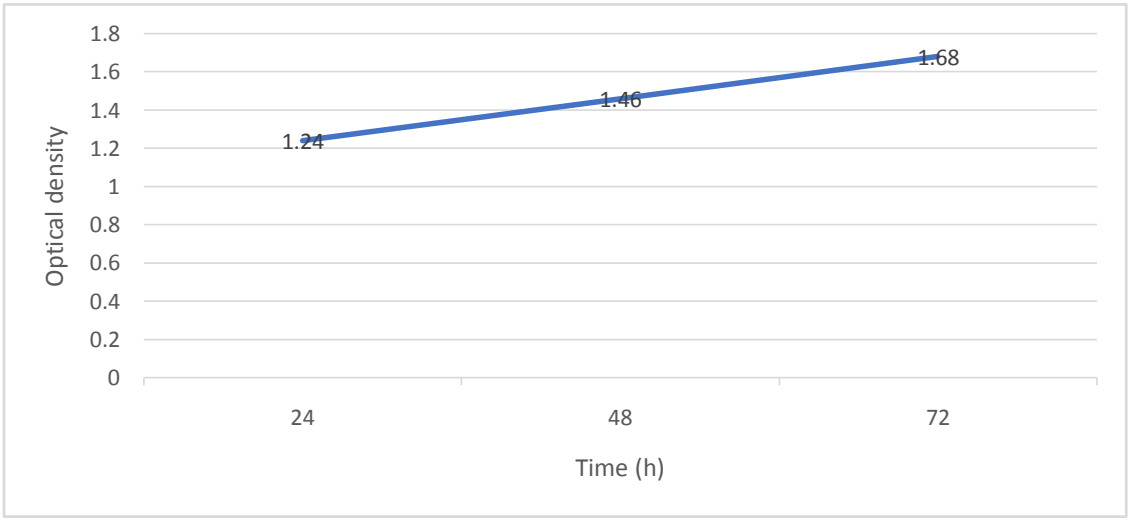

Figure: Concentration of nitrate removal at different time period

IV. CONCLUSION

From the above observation it can be conclude that the microbial population is capable to surviving at higher nitrate concentration as well as in the presence of xenobiotic compound like nitroglycerine. The organism pseudomonas aeruginosa is able to produce respiratory enzyme such as nitrate reductase and nitrite reductase that use nitrate as a terminal electron acceptor and reduce nitrate to nitrite and then ammonia and nitrogen gas by using denitrification pathway. Which can applied for bioremediation process of pharmaceutical waste effluent.

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