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Bioremediation Potential of Bacterial Isolates for Municipal Wastewater Treatment

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ABSTRACT

The potential of bacteria for the treatment of municipal wastewater was investigated in present study. Total eight bacterial isolates were used for this study that showed growth on wastewater agar medium. These isolates were identified on the basis of morphological and biochemical test and identified as *Bacillus licheniformis* NW16, *Pseudomonas aeruginosa* NS19, *Pseudomonas* sp. NS20, *Planococcus salinarum* NS23, *Stenotrophomonas maltophilia* NS21, *Paenibacillus sp.* NW9, *Paenibacillus borealis* NS3 and *Aeromonas hydrophilia* NS17. The *B. licheniformis* NW16 showed highest potential to reduce all parameter under study than other isolates except Ammonical nitrogen. *B. licheniformis* NW16 and *Aeromonas hydrophilia* NS17 showed maximum reduction (42.86%) in BOD each. *B. licheniformis* NW16 and *Paenibacillus sp.* NW9 showed 82.76% and 81.61% reduction in COD respectively. *B. licheniformis* NW16, *P. salinarum* NS23 and *Aeromonas hydrophilia* NS17 showed reduction in nitrate ranging from 17.36%-63.64%. All the isolates have potential to reduced phosphate from 17.55% -72.3%. *B. licheniformis* NW16, *Ps. aeruginosa* NS19, *Pseudomonas sp.* NS20, *Paenibacillus sp.* NW9 and *Aeromonas hydrophilia* NS17 showed reduction in TSS ranging from 42.69%-79.94%. *B. licheniformis* NW16, *Ps. aeruginosa* NS19, *Pseudomonas sp.* NS20, *S. maltophilia* NS21 and *Paenibacillus sp.* NW9 showed reduction in TDS ranging from 14%-81.4%.

Key words: Municipal wastewater, Wastewater agar medium, Bioremediation, BOD, COD.

INTRODUCTION

Water is one of the most important natural resource required to all living organisms. Its diversified uses include drinking, cooking, washing, irrigation and industrial activities (Rathore *et al*., 2014). Recently, water pollution is main problem because of uncontrolled urbanization which is due to sewage effluent disposed into water bodies and leads to the adverse effect on living organism (Tamil Selvi *et al*., 2012). Due to such problems the main global agenda is environmental management, treatment and disposal, wastes recycling, pollution control and prevention and reuse of the wastewater (Azab, 2008).

Sewage water is a complex matrix. These include high concentration of BOD, COD and high dissolved solid. The quality of wastewater is determined by analysing parameters such as COD, BOD, nitrate, TSS, TDS etc. These parameters provide crucial information of the quality of the wastewater (Loos *et al*., 2013). Wastewater is generated by residential, institutional, commercial and industrial establishments and includes household liquid wastes from baths, toilets, kitchens and sinks that are disposed off via sewers. The composition of sewage is differ widely but may contain more than 95% water with pathogens i.e. bacteria, viruses and parasitic worms and non-pathogenic bacteria. Chemical contaminants include organic particles, inorganics particles (Shannon *et al*., 2007).

Global attention has been drawn on ways to sustain the environment using microorganism to remediate environmental pollutants because physical and chemical treatment are costly and can lead to production of toxic substance (Luka *et al*., 2014). Bioremediation involves the use of microorganism to reduce or remove the pollutants from contaminated area which may lead to restoration of the original natural substance without further disruption to the local environment (Vidali, 2001; Deveraja *et al.,* 2002; Vezzulli *et al*., 2004). Mostly, the oxidized products of organic materials are CO_2 and new microbial cells. The organic matter provides energy and carbon as a nutrient source for cell growth (Chui *et al*., 2006). Bioremediation is an economical, eco-friendly and requires less expensive techniques for water pollution. But the correct microbe should be utilized in the appropriate place with the precise environmental factors (Boopathy, 2000). Therefore, the main goal of present study was to examine the ability of indigenous bacteria for bioremediation of the municipal wastewater.

Sample collection and site

Wastewater and sludge samples were collected from various places from Buldana district, India, in pre-sterilized bottle and Zip-lock plastic bag respectively according to standard procedures from American Public Health Association (APHA, 2005) and transferred immediately to the laboratory.

Isolation and identification of bacterial isolates

Wastewater and sludge samples were serially diluted and inoculated on the Nutrient agar medium separately. Morphologically different colonies were isolated and maintained at 4^0C on nutrient agar slants. The purified isolates were identified by morphological and biochemical characteristics based on Bergey's Manual of Determinative Bacteriology (Holt, 1994).

Preliminary screening of efficient bacterial isolates for bioremediation study

All bacterial isolates were inoculated on wastewater agar medium (WWA). The composition of the medium per 100 ml was 100 ml sterilized wastewater and 2% agar. All plates were incubated for 48 hr at 37^0C. Those bacterial isolates which showed growth on WWA medium were used for bioremediation studies.

Characterization of wastewater samples

Wastewater samples were characterized before and after the treatment. The parameters under study include pH, BOD, COD, Ammonical Nitrogen, Nitrate, Phosphate, TSS and TDS. All parameter were analyzed by using Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Removal efficiencies of all parameters were calculated according to the following equation:

Removal Efficiency (RE %) = C_0-RC/C_0× 100

Where, C_0=Initial Concentration before Treatment, RC= Final Concentration after Treatment

Batch culture study of bacterial isolates

Each bacterial cultures were inoculated individually in pre-sterilized 50 ml wastewater broth (WWB) i,e. only wastewater, in this 0.5% peptone

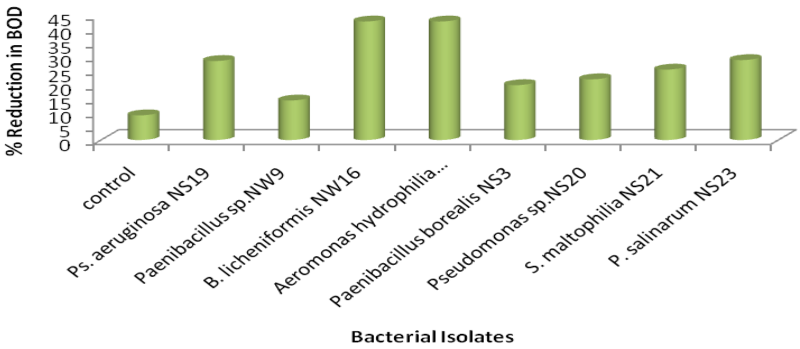

Fig.1: % Reduction in BOD by bacterial isolates

were added to enhance the growth of bacterial isolates. The flask was kept in a shaker at 120 rpm for 48 h at 37°C. The O.D. of cell suspension was adjusted to 0.5 by using sterile saline solution (0.85%) at 600 nm. Inoculum of each isolates (10%) was taken in 250 ml flask containing 90 ml non-sterile wastewater separately. Along with this, one control flask was used containing only non-sterilized wastewater. These flasks were kept in shaker at 120 rpm for 72 hr. After treatment, all samples were centrifuged at 10,000 rpm for 20 minutes at 10°C and supernatants were used for further analysis.

RESULTS AND DISCUSSION

Isolation and characterization of bacterial isolates

Total eight bacterial isolates were identified on the basis of morphological and biochemical characteristics. It includes *Bacillus licheniformis* NW16, *Ps. aeruginosa* NS19, *Pseudomonas* sp. NS20*, P. salinarum* NS23, *S. maltophilia* NS21*, Paenibacillus borealis* NS3*, Paenibacillus* sp. NW9 and *Aeromonas hydrophilia* NS17.

Batch culture study of bacterial isolates

Total 44 bacterial isolates were isolated on nutrient agar medium. Out of these, 8 bacterial isolates showed growth on WWA medium. The organic matter contained in the wastewater provides a substrate for these isolates. These isolates were used for bioremediation studies by batch experiments.

The initial pH of the sample was slightly acidic whereas it was slightly alkaline after treatment. The control showed negligible change in pH (data is not shown here). Degradation of proteins and amino acids present in wastewater was converted into ammonia that increases the pH of sample to slightly alkaline. The change in pH of wastewater suggests that there has been activity of microorganisms which degrade organic matter.

Biochemical oxygen demand (BOD) is a measure of the oxygen required to microorganisms for the decomposition of waste material. The initial BOD of the samples was higher than the permissible limit. High BOD indicates high amount of organic

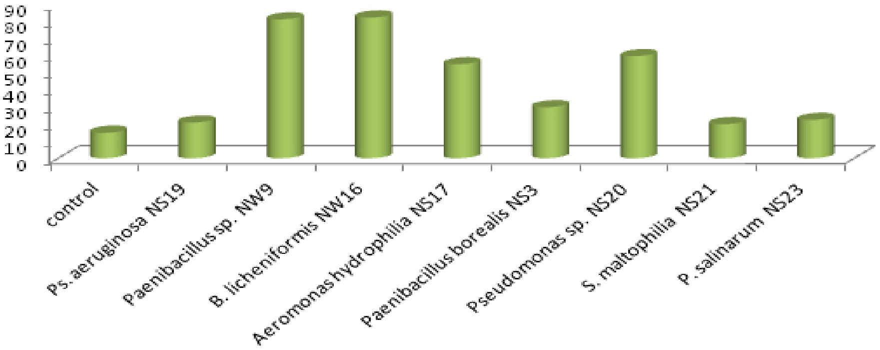

Fig.2: % Reduction in COD by bacterial isolates

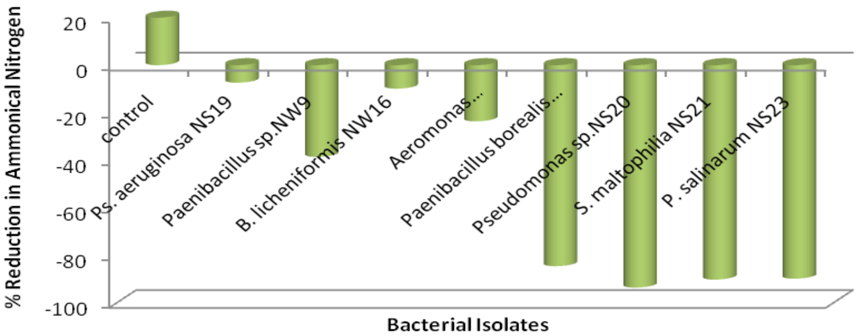

Fig. 3: % Reduction in Ammonical Nitrogen by bacterial isolates

matter, it lead to oxygen depletion and creates anaerobic conditions which would result in reduction of diversity and distribution of aquatic fauna. Organic matter will support anaerobic action leading to the accumulation of toxic compounds in water bodies (Goel, 1997). The maximum percentage removal of BOD in 72 hours was observed by *B. licheniformis* NW16 and *Aeromonas hydrophilia* NS17 i.e. 42.86% each followed by *P. salinarum* NS23, *Ps. aeruginosa* NS19*, S. maltophilia* NS21*, Pseudomonas* sp. NS20, *Paenibacillus borealis* NS3 and *Paenibacillus* sp. NW9 with 28.91%, 28.57%, 25.46%, 21.96%, 19.85% and 14.29% respectively whereas control showed only 8.93% (Fig. 1). Similar results were observed by Shrivastava *et al*., (2013) and Prasad and Manjunath (2011) and found that *B. subtilis* and *Pseudomonas aeruginosa* has BOD and COD reduction potential while studying on bioremediation of Yamuna water and lipid rich wastewater respectively. Vasconcellos *et al*., (2009) reported *B. licheniformis* for biodegradation of cassava processing wastewater whereas Ravi Kumar *et al*., (2013) reported 36.41% BOD removal efficiency by *B. licheniformis* for bioremediation of sewage.

Chemical Oxygen Demand (COD) test is the best and rapid method for estimation of organic matter present in the wastewater sample. In our study it was observed that all isolates showing reduction in COD after 72 hr (Fig. 2). *B. licheniformis* NW16 showed maximum reduction in COD (82.76%) followed by *Paenibacillus* sp. NW9, *Pseudomonas* sp. NS20, *Aeromonas hydrophilia* NS17, *Paenibacillus borealis* NS3, *P. salinarum* NS23, *Ps. aeruginosa* NS19 and *S. maltophilia* NS21 with 81.61%, 60%, 55.3%, 30%, 22.5%, 21% and 20% respectively and compared with control which showed 14.94% reduction in COD. In biodegradation, bacteria uses organic compound as a substrate for their growth and development (Chin *et al*., 1995). These bacteria are capable of producing a wide variety of enzymes that can degrade complex organic compounds into CO2 and water present in the wastewater (Claxton and Houx, 1995; Chin *et al*., 1995). The bacterial species present in the wastewater has no significant effect on removal of BOD and COD as observed in case of control. However, our isolates showed promising results. Similar results were observed by Gaikwad et al., (2014) and Zhao et al., (2009) found that Pseudomonas sp. and Bacillus sp. were able to

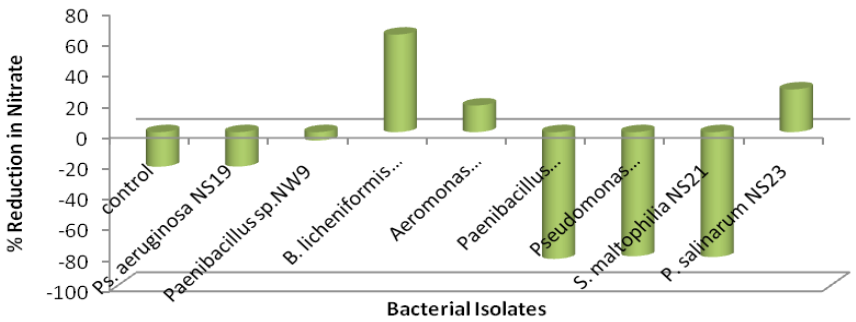

Fig. 4: % Reduction in Nitrate by bacterial isolates

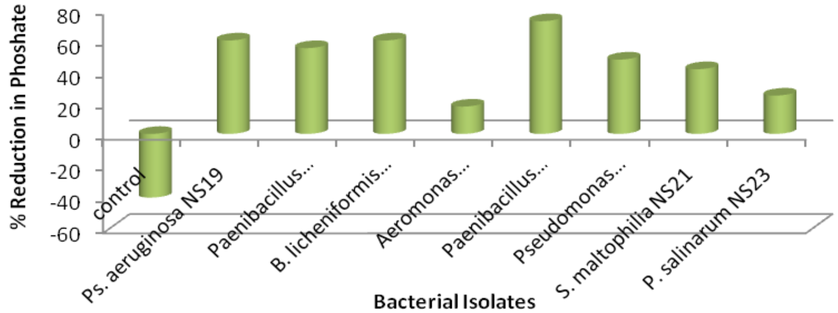

Fig. 5: % Reduction on Phosphate by bacterial isolates

reduced COD and BOD. Mazzucotelli *et al*., (2014) reported use of Stenotrophomonas for degradation of dairy wastewater and Ji-hong *et al*., (2008) also reported COD reducer Aeromonas sp.

In the present study, none of the bacterial isolates showed reduction in Ammonical nitrogen (Fig. 3). The increase in the concentration of Ammonical nitrogen was because of degradation of protein and nitrogenous compounds into ammonia.

The nitrate concentration prior to treatment was very high than permissible limits. Nitrate is one of the source for eutrophication of water. In the present study, the maximum reduction in nitrate was showed by *B. licheniformis* NW16 (63.64%), *P. salinarum* NS23 (27.87%) and *Aeromonas hydrophilia* NS17 (17.37%). It was observed that the increase in the concentration of nitrate in case of *Ps. aeruginosa* NS19, *Pseudomonas* sp. NS20, *S. maltophilia* NS21, *Paenibacillus borealis* NS3 *and Paenibacillus* sp. NW9 suggests the process of nitrification whereas decrease in nitrate concentration in case of *B. licheniformis NW16, P. salinarum NS23*, *Aeromonas hydrophilia* NS17 showed denitrification process that is conversion of nitrate into the molecular nitrogen (Fig. 4). Rajakumar *et al*., (2008) reported that *Bacillus* sp. and *Pseudomonas* sp. were most efficient for nitrate reduction. In our study, *Bacillus* sp. showed reduction in nitrate whereas *Pseudomonas* sp. showed contrasting result.

The phosphate is one of the most serious environmental problems because of its contribution to the eutrophication process of lakes and other natural waters. It occurs in natural water, wastewater, sediments and sludge. The possible entry of this ion into aquatic environment is through household sewage. The reduction showed by *Paenibacillus borealis* NS3 (72.3%), *Ps. aeruginosa* NS19 and *B. licheniformis* NW16 (59.98%) each, *Paenibacillus* sp. NW9, (55.06%), *Pseudomonas* sp. NS20 (47.69%), *S. maltophilia* NS21 (41.54%), *P. salinarum* NS23 (24.61%) and *Aeromonas hydrophilia* NS17 (17.55%). The control showed no removal in phosphate concentration (Fig. 5). Similar results were observed by Krishnaswamy *et al.,* (2011) and found that the *Bacillus* sp RS-1 and *Pseudomonas*

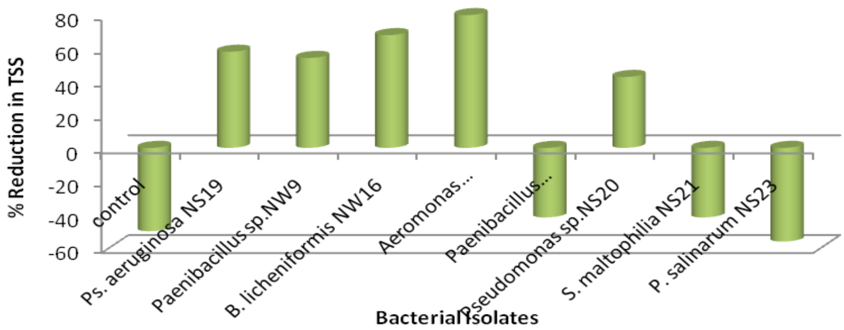

Fig. 6: % Reduction in TSS by bacterial isolates

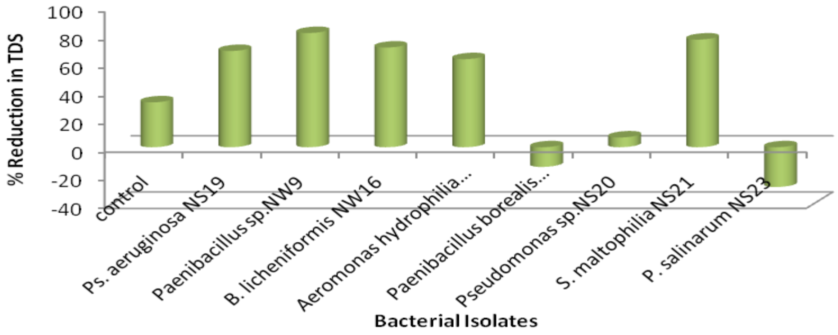

Fig. 7: % Reduction in TDS by bacterial isolates

sp. YLW-7 were found to be efficient in phosphate reduction.

High amount of suspended particles has detrimental effects on aquatic flora and fauna and reduce the diversity of life in aquatic system and promote depletion of oxygen (Karthikeyan *et al.,* 2010). The results from Fig.6 suggested that the maximum reduction in TSS was showed by *Aeromonas hydrophilia* NS17 (79.94%) followed by *B. licheniformis* NW16 (67.88%), *Ps. Aeruginosa* NS19 (57.88%), *Paenibacillus* sp. NW9 (54.15%), *Pseudomonas* sp. NS20 (42.69%) whereas *Paenibacillus borealis* NS3, *P. salinarum* NS23, *S. maltophilia* NS21and control were unable to reduce TSS.

Total dissolved solid refers to all dissolved materials present in the wastewater. Discharge of wastewater with a high TDS level would have adverse impact on aquatic life and reduces crop yields if used for irrigation. In this study most of the isolates showed reduction in TDS. The maximum reduction in TDS was showed by *Paenibacillus* sp. NW9 (81.4 %,), *S. maltophilia* NS21 (76.74%), *B. licheniformis* NW16 (71.08%), *Ps. aeruginosa* NS19 (68.6%), *Aeromonas hydrophilia* NS17 (62.79%), control (32%), *Pseudomonas* sp. NS20 (6.98%) whereas *Paenibacillus borealis* NS3 and *P. salinarum* NS23 were unable to reduce TDS (Fig. 7). Tomar and Mittal (2014) also found that *B. subtilis* has potential for reduction of TSS and TDS.

CONCLUSION

In the present study, total eight bacterial isolates namely *B. licheniformis* NW16, *Ps. Aeruginosa* NS19*, Pseudomonas* sp. NS20, *P. salinarum* NS23*, S. maltophilia* NS21*, Paenibacillus borealis* NS3, *Paenibacillus* sp. NW9 and *Aeromonas hydrophilia* NS17 were isolated and used for bioremediation of municipal wastewater. These isolates were showed degradation of organic matter in term of BOD, COD, nitrate, phosphate, TSS and TDS. Hence, these isolates may be used for bioremediation of municipal wastewater to control water pollution.

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