

Investigation of the Potentials of Local Strains of *Pseudomonas* for Aquaculture Water Treatment

Alozie, A.; John, C. I.; Hanis, B.; Egbulefu, C. S.; and Mbambam, F.

National Biotechnology Research and Development Agency (NBRDA), Abuja, Nigeria.

Corresponding author: josmarachi@yahoo.com

DOI: <https://doi.org/10.62154/ajasfr.2025.018.010653>

Abstract

The need for human and animal food production cannot be over emphasized. Aquaculture and related activities have become an important sustainable business in Nigeria. Water pollution rates has increased in modern time owing to the various chemical usage. The receiving water bodies therefore constitute potential source danger to both human and animal. Several metabolites of the bacteria have ability to cause detoxification of polluted water. There is need to develop indigenous bacteria consortia for application in the treatment of waste water bodies in order to reduce importation and to earn local earnings. Some indigenous *Pseudomonas* strains were isolated from waste soil sites using standard microbiological techniques including pour-plating serially diluted 1g of the soil sample in 9ml of sterile peptone water. The basic features of the developed discrete colonies were studied, sub-cultured and then the purified isolates; PM05, PM78, PM13 and PM56 were challenged by growing them respectively with waste water. Specifically, the $\text{NH}_4^+\text{-N}$ content decreased from 230mg/L to a range of between 0.5 to 12 mg/L. While NO_3^-N decreased from 25 mg/l to between 5.3 to 14.6mg/L. $\text{NO}_2\text{-N}$ decreased from 12mg/L to between 1.3 to 5.7 mg/L. and available/total N decreased from 235 to between 103 mg/L respectively. The results indicated that the strains generally have the potentials to remove nitrogen through the metabolic pathway of nitrogen assimilation which may make them fit for further development for bio-remediation purposes. Also, the results generated will add to the previous knowledge that such important strains can be obtained from the local soils and sediments in Nigeria.

Keywords: *Pseudomonas*, Bioremediation, Detoxication, Waste Water, Metabolites.

Introduction

Feeding livestock according to their requirement and avoiding wastage is very important in exploiting the production potential for economic growth and sustainability since feed costs are the dominant cost and accounts for more than 70% of the cost of production. Therefore, new technology for production of forages i.e., hydroponics fodder production system is now a hope for sustainable livestock production. Hydroponic fodder is a viable way to ensure the long-term availability of high-quality fodder for livestock. Hydroponics have been produced from several cereal crops such as barley (Naik *et al.*, 2015), oats, wheat (Snow *et al.*, 2008); sorghum, alfalfa, cowpea (Al-Karaki and Al-Hashimi 2012) and maize (Naik *et al.*, 2011; Naik *et al.*, 2012). The fodder can be produced in a short duration (6-10

days) and all year around. The hydroponics fodder present various benefits for animal health. It is rich in protein, vitamins, fiber, and minerals (Girma and Gebremariam 2018). Feeding hydroponically produced fodder enhance the digestibility of the ration's nutrients (Hassen and Dawid 2022). Hydroponic fodder production is an economic solution particularly where the conventional production of green fodder is limited or unavailable. This technology put forward a solution to address the shortage of forage production caused by the scarcity of green feed in dry seasons and urban areas (Ndaru *et al.*, 2020). It is one of the important agricultural techniques used nowadays in different countries.

The rabbit production is adaptable to wide range of production systems from backyard to commercial multi-tier cage rearing systems. Rabbit production would boost the income of the farmer and could improve human nutrition. Rabbit production with appropriate technologies can play an important role in scaling up the production traits and thereby economic benefits to the farmers. Quality green fodder is an important input for rabbit production. It is inevitable to produce green fodder by alternative method for feeding livestock including rabbits.

The need for human and animal food production cannot be over emphasized. Aquaculture and related activities have become an important sustainable business in Nigeria. Aside being a notable source of income at all levels, it is a major source of proteins for both human and animals (Erinsakin *et al.*, 2020). Aquaculture practice has been on for long. However, the need for modern and expansion to cope with increasing population cannot be over stated.

Expansion of aquaculture especially fish production goes beyond depending on the natural ponds and lake to including artificial pond systems that can be optimized (Fig.1). Among the common challenges to aquaculture production on large scale aside disease control is the need to constantly monitoring the water production system for the good health of the animals (Ambust *et al.*, 2021). Nitrite and Nitrate are associated with aquaculture production.



Fig. 1: Typical aquaculture system in Nigeria

Caring for pond fish include the science of water quality management and the pond nitrogen cycle which makes it imperative to monitor pond water to make sure that the fish stay healthy. While pond water quality is important to the health of all the animals therein, testing often can be expensive, complicated or scary. Chemical over-use can mess up the system by becoming obsessive to make sure the water is pristine and perfect. (Cao *et al.*, 2021). The natural course of completion of the nitrogen cycle produces Nitrite and ultimately Nitrate. While Nitrite interfere with the blood of fish and other aquatic lives leading to death, Nitrate at moderate concentrations has not been attributed to serious life-threatening case (Chen, *et al.*, 2022). However, with chronic, high levels of nitrate, fish become weaker, slower growth, and increased illness overall. This is because high nitrate levels represent inferior water quality and this causes stress, making fish more vulnerable to diseases. Nitrates should always be less than 80 ppm in an ornamental pond.

To reduce nitrates, plants can be added, water changes can be performed, or algae growth can be encouraged. Nitrate is the primary source of dissolved inorganic nitrogen in wastewater treatment plant effluent (Deivakumari *et al.*, 2022). High nitrate concentration in the surface water may cause eutrophication and harmful algal blooms. Meanwhile, excess nitrate has a serious impact on human health, such as methemoglobinemia and carcinoma. Thus, it is of important significance to remove nitrate from contaminated water. Commonly applied methods for nitrate removal include ion exchange, adsorption, reverse osmosis, electrodialysis, catalysis, and biological denitrification (Gao *et al.*, 2022). However, there are some demerits to the use of these methods which include the very high cost of

initial set up as well as the formation of byproducts after treatment. The process is also deemed expensive beyond the reach of many that would have wanted to use the method. Therefore, biological method of removing these compounds and elements is being held in high esteem owing to their many advantages (Gutiérrez *et al.*, 2020). This method is environmentally friendly, as well as cost-effective. It is however well known that conventional biological nitrogen removal contains two stages: denitrification follows nitrification to accomplish ammonium removal. However, it is difficult to implement such a system in natural environments because nitrification needs aerobic conditions while denitrification requires anoxic conditions.

Many aerobic bacteria have been isolated with potential to cause denitrification at aerobic level (Haider *et al.*, 2021). They include many pseudomonas which has been used for aquaculture remediation with successful outcomes. With advancement and urbanization, more complex wastes are generated that are adding more pressure on the receiving artificial water bodies where traditional aquaculture practice take place (Kalaimurugan *et al.*, 2021). Within the confine of intensive aquaculture, inferior quality-feeds use for the fish production sometimes dissolves in the water thereby producing end-product like Nitrite and Nitrates. Also, owing to the limitation of the conventional sewage treatment process, the effluent of municipal wastewater treatment plants still contains relatively high nitrate levels (Karadžić *et al.*, 2021).

Therefore, the need to adopt biological methods that thrives in aerobic environment cannot be over emphasized as effluent bioremediation method. Due to their advantages of easy implementation, economic benefits and high efficiency, biological methods are used more commonly than physical and chemical methods in the sewage treatment industry (Kumar *et al.*, 2021). One of the most common nitrogen sources in wastewater, nitrate, can be removed by microbial reduction in the following two ways: (1) release by denitrification in the form of N_2 , N_2O and other gases or (2) conversion to ammonium by dissimilated nitrate reduction (DNR) or to organic nitrogen by assimilated nitrate reduction (ANR) (Robertson *et al.*, 2020).

Several metabolites of the bacteria have ability to cause detoxification of polluted water. Comparatively, many bacteria such as *Pseudomonas* with the capability of NO_3^- removal under aerobic conditions have been documented (Nejad *et al.*, 2020). These bacteria are found in the environment as an abundance natural resource. The potentials of *Pseudomonas* to bioremediate chemicals have been reported. Biological Nitrogen removal using *Pseudomonas* strains is of great interest for wastewater treatment. For the application of highly-efficient bacteria, the strategy of bioaugmentation is frequently adopted by adding selected strains/mixed cultures to the system to improve the removal of specific compounds (Rai, *et al.*, 2020). There have been two major prerequisites to the success of bio-augmentation success; one is colonization ability of the Bacteria cultures and the maintenance of degrading-efficiency for the inoculated bacteria. The basic concept of bioremediation is that microorganisms including *Pseudomonas* can use pollutants as carbon and/or nitrogen sources and completely mineralize them into carbon dioxide and

water, or at least convert them into non-toxic secondary decomposition products (Setlhare *et al.*, 2020). This process is not only safe and clean, but certain intermediates can also be used as raw materials for the synthesis of other products due to the unparalleled chemo-selectivity of enzymes. Therefore, bioremediation is an ideal method for removing environmental pollutants. Also, mineral fertilizer is a double-edged sword creating a number of problems inherent to its production and use. First, N fertilizer production by the Haber-Bosch process is energy intensive and currently still driven by fossil fuels, resulting in greenhouse gas emissions (Medić *et al.*, 2022). Second, in addition to the growing price of fertilizer, a looming problem is excessive nitrogen use that can lead to nitrate leaching into ground water or, depending on soil pH, redox potential, and microbial activity, to NH_3 or N_2O emissions causing N losses and environmental pollution (Muthukumar *et al.*, 2022). Some *Pseudomonas* strains have been found to fix Nitrogen in the soil thereby improving the soil fertility (Suzuki *et al.*, 2021).

In Nigeria, there is still scarcity of knowledge about the application of indigenous strains of microorganisms for biotechnology processes such as bio-remediation. The present lack of standardized local strain of *Pseudomonas* that can efficiently utilize wastes is a hinderance to the use of biological methods for addressing waste management. The readily adaptable local strains to local environment have been reported to be an advantage to full utilization of microbial agents (Varjani *et al.*, 2020). The strains of *Pseudomonas* used for bioremediation of pond water are known to be safe in terms of environmental concerns in that they are neither pathogenic nor carry pathogenic genes. The knowledge gleaned from the result of this study will contribute to further enhance the search for local strains of microbial resources for this application. This would further reduce over-dependence on exotic strains for similar purposes as well as the use of chemicals which are often considered as not environmentally friendly. Therefore, the aim of this study was to isolate, and screen wild *Pseudomonas* species with bioremediation abilities as a bio-control of aquatic water.

Materials and Methods

Source of Bacteria and Isolation Protocol

The *Pseudomonas* strains have been reported to be highly efficient in the degradation and bio-conversion of organic waste in the processes now applied as bio-remediation owing to several intrinsic factors such as possession of active enzyme activity (Abo-Amer *et al.*, 2022). The strains used in this research were isolated from sewage water samples, soil sediments, humus soil and soil sediments in and around the Federal Capital Territory (FCT), Nigeria. Serial dilutions of the respective sample were prepared as described by (Sahinkaya *et al.*, 2021). Plates *Pseudomonas* agar media were inoculated with the prepared dilutions (10^{-4} and 10^{-5}) and incubated at 37°C for 24-48 h.

Morphological and Colony Characterization of Isolates

The resultant bacterial isolates obtained from the respective samples were examined for their Gram reaction, colony morphology, fluorescence and cell shape (Kong *et al.*, 2020).

The microscopic observations in oil emersion were recorded on the basis of their shape, size, color, opacity and mucosity. Colony characterization was based on their shape, size, color, opacity and mucosity. Individual bacterial isolates were streaked on the media plates and incubated at 35°C for 5days to record colony characteristics.

Identification of Selected Isolates based on Biochemical Characterization

Basic biochemical test including, Catalase test, Starch hydrolysis, Triple sugar iron agar (TSIA), Indole production, Hydrogen sulphide production, Methyl red test (MR test) and Voges-Proskauer test (VP Test) were conducted to identify the selected isolates as described in Bergey's Manual of Determinative Bacteriology (Liu, *et al.*, 2022).

Evaluation of the Potentials of Isolates

The aquaculture water used in the current experiment were obtained from households rearing fish and fish farms. The water and the effluent from such fish farms were obtained in clean containers. The respective water sample was filter-sterilized into make a volume of 250mL in a 500mL flask each. Each pure strain of the *Pseudomonas* was first cultured in LB medium for 12 h, then it was inoculated into the flask containing the filter-sterilized water with 10% inoculation ratio. The Culture was supplemented with sodium citrate (at optimized concentration) as carbon source and cultivated under the optimal conditions. A sample each was left uninoculated to serve as control. Concentrations of NO_3^- -N, NH_4^+ -N, NO_2^- -N and TN were determined periodically. All experiments were performed in triplicate (Braker and Tiedje, 2023).

Results and Discussion

Isolation and Characterization Bacteria Isolates

Based on the expected characteristics of *Pseudomonas*, the bacterial isolate was identified and were found to be *Pseudomonas* (Fig. II). However, *Pseudomonas cepacian* PM05, *Pseudomonas putida* PM78, *Pseudomonas aeruginosa* PM13 and *Pseudomonas putida* PM56 were thereafter stored in 20% glycerol at 4°C till further needed. One reference strain AB74K obtained from the department of Microbiology, Ahmadu Bello University was maintained in the same way.

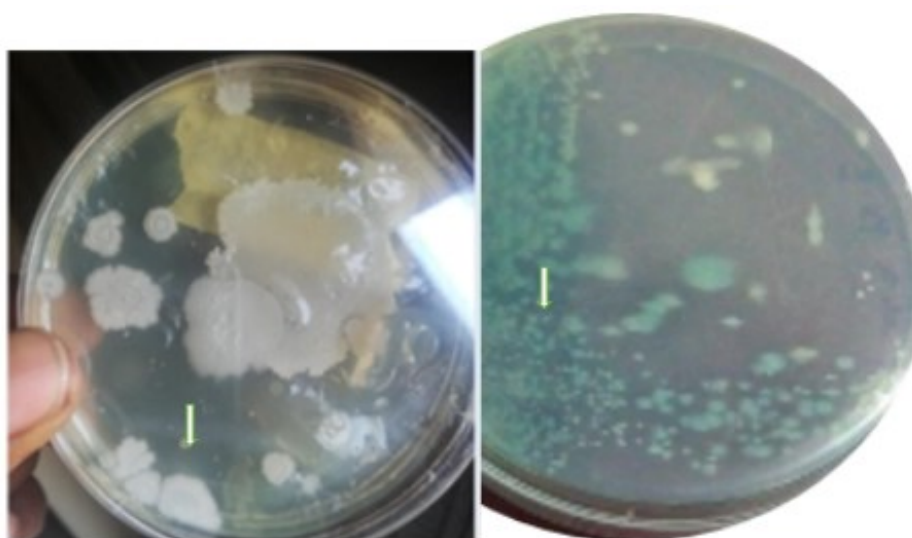


Fig. II: Pseudomonas colonies on Agar plates

As it could be seen from this research, the presence and abundance of Pseudomonas in the environment have widely been reported. Generally, Pseudomonas has been reported to be a group of bacteria found in soil, water, skin flora and most man-made environments throughout the world and in all habitats (Canfield and Glazer, 2010). They may be responsible for infectious diseases, generalized inflammation and sepsis. It can also be found on medical equipment. Pseudomonas is well known to be animal and plant pathogen. It is also reported to cause food spoilage. Several species have also been reported to be involved in the cycling of organic matter in nature (Padhi *et al.*, 2013).

Degradation Potentials of Isolates

Table 1: The degradative N-related components of Pond by the isolates

Isolate code		Removal efficiency (before and after treatment (mg/l))				
Component	Time	Bacteria isolate (code)				
		PM05	PM78	PM13	PM56	AB74K
$\text{NO}_3^- \text{N}$	Before	25	25	25	25	25
	After	5.3	14.6	13.9	11.4	20.5
$\text{NH}_4^+ \text{-N}$	Before	230	230	230	230	230
	After	0.5	10	11	5	12
$\text{NO}_2^- \text{N}$	Before	12	12	12	12	12
	After	1.3	4.2	5.7	3.8	4.3
TN	Before	235	235	235	235	235
	After	103	114	123	131	210

From the Table I, specifically, the $\text{NH}_4^+ \text{-N}$ content decreased from 230 to a range of between 0.5 to 12 mg/L. While $\text{NO}_3^- \text{N}$ decreased from 25 mg/l to between 5.3 to 14.6mg/L. $\text{NO}_2^- \text{N}$ decreased from 12mg/L to between 1.3 to 5.7 mg/L. and available/total N decreased from

235 to between 103 and 210mg/L respectively. The results showed that the bacteria strains had remarkable effect on the removal of the elements from the waste materials. However, PM05 had the highest ability having reduced the NO₄-N from 230mg/mL to 0.5mg/L (Fig. I and II). The ability of microbial strains in bio-conversion of wastes is strain-dependence. Strain PM05 must have had some intrinsic advantage such as extracellular enzyme production and possession of permeable membrane for the release of same. Numerous studies have shown that *Pseudomonas aeruginosa* is a powerful platform for the remediation of environmental pollution with organic chemicals due to its diverse metabolic network and its ability to secrete biosurfactants to make hydrophobic substrates more bioavailable, thereby facilitating degradation. This was earlier reported by Fanghui *et al.*, 2024 in the review on "Bioremediation of environmental organic pollutants by *Pseudomonas aeruginosa*: Mechanisms, methods and challenges".

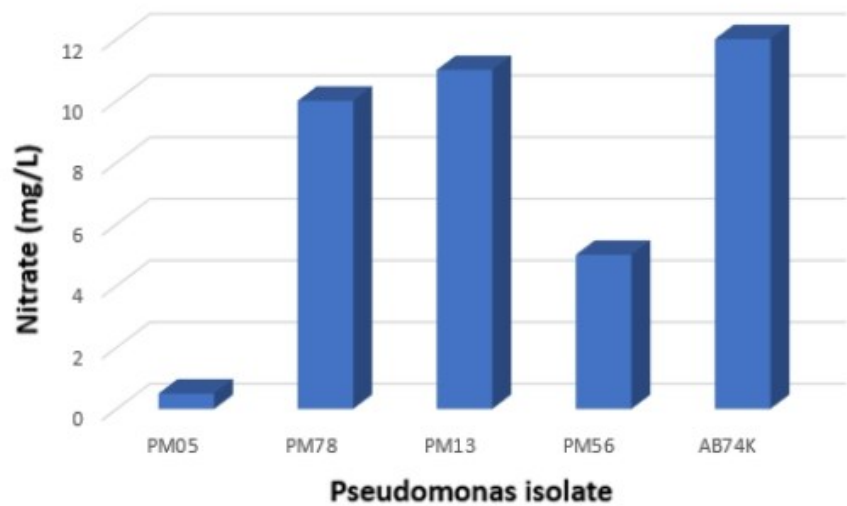


Fig. III: Nitrate Removal potential of isolates

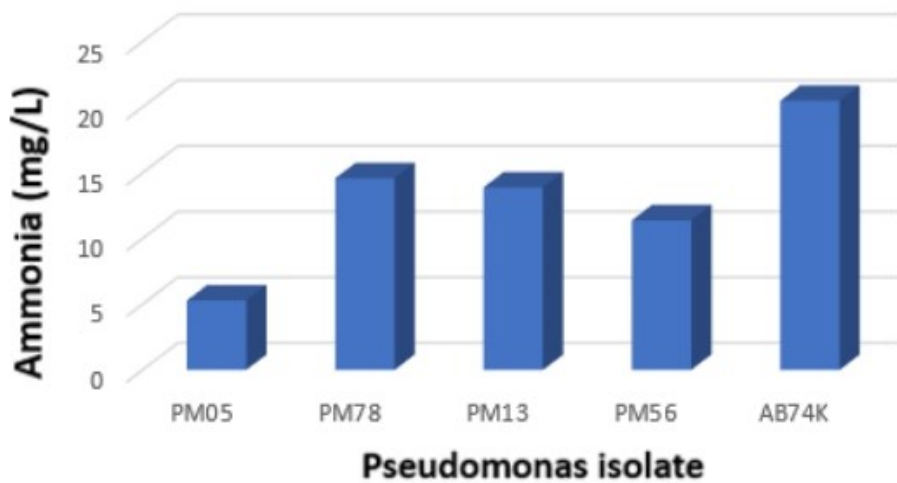


Fig. IV: Ammonia Removal potential of isolates

This agrees with the work of Huang *et al.*, (2015) that the efficiency of *Pseudomonas* was observed by the reduction of Nitrate from 260mg/mL to 18mg/mL. This suggest that the strain can be used to develop a single-stage wastewater treatment process for a simultaneous removal of organic matter and $\text{NH}_4^+\text{-N}$, which has excellent application prospects in domestic and aquatic water system (He *et al.*, 2016). Nitrate is an end product of nitrification and feed for denitrifying heterotrophs. It is negligibly toxic compared to ammonia and nitrite. It is toxic in aquaculture system when its concentration is beyond 200 mg l⁻¹. Nitrate on reduction converts to nitrogen or back to ammonia. The pathway which leads to the formation of gaseous nitrogen, is the most ideal in a culture system, as in the ammonia production which otherwise tends to further increase the toxicity of the system. Such organisms like *Pseudomonas* isolate in this work can also be mass-produced and introduced in to the system. According to Lei *et al.*, (2016), under low oxygen conditions nitrate nitrogen ($\text{NO}_3\text{-N}$) can be used as alternative electron acceptor by heterotrophs. Present investigation also reveals that initial nitrate levels were very low in the polluted water. This is due to poor nitrification (as a result of poor nitrifiers' population, poor dissolved oxygen and high COD, which slowly improved because of nitrifier's inoculation and aeration).

Bioaugmentation technology refers to the methods of improving wastewater treatment effect by adding microorganisms with specific functions in the biological treatment system, which promotes the removal of certain pollutants in polluted water (Qu *et al.*, 2022). The characteristics of nitrate nitrogen removal by the isolate sin this work using shaking flask medium culture conditions had earlier been documented (Ren *et al.*, 2021). However, few studies concerned the removal of nitrate nitrogen by using indigenous, wild *Pseudomonas* strains in aquatic water system. Therefore, this potential application of the local isolate was investigated. Therefore, strain PM05 has potential application in the advanced treatment of aquaculture wastewater treatment plant effluent. Nevertheless, subsequent experiments should be carried out to discuss the strategies of bioaugmentation and the survival capability of all the other in the practical wastewater treatment system as well as optimize the conditions as reported by Qu *et al.*, (2022). Some researchers recognize some of the problems bedeviling aquaculture development in Nigeria and found out a number of them which included management of wastes (Shi *et al.*, 2022). Others include high quantity of fish seed, energy and water quality related problems arising from skills gap in the industry. The ability of the isolated *Pseudomonas* strains to reduce the Nitrates suggest that it could be further studied for consideration in bioremediation formulation. By this, the aim of the study which was to isolate, and screen wild *Pseudomonas* species with bioremediation abilities as a bio-control of aquatic water would be achieved.

Conclusion and Recommendations

Going by recent data, Nigeria is the second-largest aquaculture producer in Africa, even though it also has a high demand and preference for fish by the consumers. However, the

role and potential of aquaculture to achieve goals for improving smallholder income, dietary diversification and women's empowerment have yet to be realized owing to some bottlenecks along the production chain. One of these is the overall cost of production and management system. An 18-month study focused on identifying aquaculture sector bottlenecks for inclusive growth, based on fish production, consumption and value chain models that have high potential to positively impact smallholder income, nutrition, youth employment and women's empowerment at scale was instituted sometimes back.

The use of natural remediation methods to remove contaminants from waste water is becoming more popular. One of the main purposes of waste water treatment is to reduce nutrient such as nitrate and phosphate level in effluent to a protective level of the receiving water body. However, treatment of aquaculture water system in order to maintain high quality hygiene and productive fishing activity uses chemical that could be replaced with biological system. This was the focus of this research. By using microorganisms such as *Pseudomonas* strains, the level of Nitrates could be kept at minimal limit thereby preventing the adverse effects their presence could cause in the water. This may also translate to saving cost and expansion of the business. Moderate cost and readily available techniques can also spur Nigerian aquaculture sector, and others members of the populace to up the opportunities toward increasing jobs especially among the rural areas and smallholders.

The results from this research indicated that the strains generally have the potentials to remove nitrogen and phosphorus through the metabolic pathway of nitrogen assimilation and phosphorus accumulation which may make them fit for further development for bio-remediation purposes. Also, the results generated will add to the previous knowledge that such important strains can be obtained from the local soils. Inoculation of aquatic waste water with isolated and screened strains of *Pseudomonas* with excellent bioremediation capacity and removal efficiency can potentially be a solution to the problem associated with current water treatment system where chemical that are not environmentally friendly are used.

From this study, the following are thereafter recommended;

- Further research on the intrinsic abilities of the high-performance strain (PM95) for possible improvement;
- Upscaling of the bio-remediative abilities to include other sources of pollutants;
- Design of small scale pilot plants to further test for the activities;
- Government at all levels should formulate policies to include the adoption of microbes in the bio-remediation of wastes. This may include construction of modern bioaugmented system for pollutant removal using biological system and can spur the use of the principle for selection of inoculated strains for bioaugmentation globally.

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