
Biopurification of Waste Water by Azolla: A Review

Ishita Paul ^{1*}

^{1*} Assistant Professor, Dept. of Zoology, Triveni Devi Bhalotia College, Raniganj, W.B: 713347, West Bengal, India. Email: ishitapaul88@gmail.com

Abstract

Water Pollution is one of the major threats of today's civilization. Around 2.3 billion people worldwide live in areas with chronic water shortage (WHO, 2005). Therefore, there is an urgent need to recycle the sewage water from various sources like coal and petroleum refinery, poultry farm, kitchen grey water etc., which can be used for our agricultural or aquaculture industry. This will also minimize the effect of the polluted water that goes back to the environment. In this review we studied the major promising purification capability of Azolla fern that purifies wastewater from different sources. As this fern can double its size within a very short period of time, it is a very crucial biological tool for wastewater purification. It also has some medicinal values. In this review we studied the noticeable removal or decrease of nitrite and phosphorous by 71.4% and 68.65% respectively in case of secondary effluents. The nitrogen fixation property of Azolla is because of its symbiosis with a nitrogen fixing bacteria called Anabaena. It has also been studied that the cultivation of Azolla in wastewater shows high and remarkable potentiality of COD removal by 98.8% in 28 days in case of petroleum refinery wastewater. So, this biopurification process by Azolla is very useful now a days to reduce the toxicity of wastewater.

KEYWORDS: Azolla, biopurification, wastewater

INTRODUCTION

Environmental contamination has increased as a result of fast population growth, urbanisation, and industrialisation (CPCB, 2008; Arora et.al. 2009; Sood et al., 2011; Sachdeva and Sharma, 2012). Herbicides, insecticides, and inorganic pollutants such as trace elements, heavy metals, and metalloids are released into the soil, water, and air, among other parts of the earth. Irrigation water contains pollutants that make agricultural soils salty and so unusable. Intensified irrigation is required in modern agriculture. As a result, groundwater resources have been overused, the water table has dropped, and many places are now experiencing drought. The world will eventually experience a situation similar to a war, with conflicts over the sharing of water between various powers.

The rise of agriculture and changes in land usage have had a significant impact on India, which once held a treasure trove of wetlands, natural water purifiers, and wildlife (Bassi et al., 2014). Artificial wetlands have been created as a result of the

decline of natural wetlands, which are capable of digesting pollutants due to their self-regulating nature. Due to its efficiency and low cost, the use of built wetlands for wastewater treatment has grown in importance since 1950 (Vymazal, 2010). These ecosystems were created with the intention of improving the quality of point and non-point sources of pollution. They have proven to be successful in treating organic pollutants that are still present.

BIOPURIFICATION

One of a wetland's most significant ecosystem services is biopurification, which also serves a restorative purpose in artificial wetlands (MA, 2005). Biopurification, one of the most cutting-edge and economical methods utilised in wastewater treatment, is the use of biological intervention to mitigate the harmful effect induced by the contaminants (Prasad and Singh, 2011). Its usage in wastewater treatment has been extensively debated due to the bioremediation activities carried out by the created wetlands, which are referred to as a green alternative to conventional technologies (Ranalli and Lundholm, 2008). Utilizing macrophytes to remove nutrients from wastewater has been documented and debated repeatedly (Fisher, 1988, DeBusk et al, 1989; Oron, 1990). Macrophytes like water hyacinth, parrot feather, duck weed, reed, and Azolla are frequently employed. Azolla is a floating object.

AZOLLA'S SIGNIFICANCE IN BIOPURIFICATION

Lamarck recognised the genus Azolla in 1783. The Greek words azo, which means to dry, and allyo, which means to kill, were combined to create the name Azolla (Jaeger, 1978). It has a number of benefits, but its ability to grow quickly is its most significant one, earning it the nickname "super plant" (Wagner, 1997). The plant's need for nitrogen is met thanks to the symbiotic nitrogen-fixing cyanobacteria *Anabaena azollae* that live inside the cavities of its leaves. In return, the fern offers the endosymbiont a safe environment and a source of carbon in the form of sucrose (Peters & Meeks, 1989). The organism's ability to trap nitrogen makes it play a significant role in agriculture through enhanced rice production.

The focus of the current study is on how well Azolla removes nitrogen, phosphorus, and carbon from secondary treated municipal wastewater for use in created wetlands. Azolla is more effective at removing nutrients from wastewater compared to other often employed ferns (duckweed, pennywort, cattail, bulrush, and water hyacinth). This is due to the fact that the typical N to P ratio in most plants is 10:1. When employing popular marophytic plants, phosphorus will typically persist even after nitrogen is removed. Azolla is unique in that it has the ability to repair N₂, which allows it to eliminate phosphorus even after nitrogen has been absorbed. Therefore, phosphorus removal that is more effective can be sought out. Additionally, they may

be cultivated in aquatic media and have a quick time of doubling as long as the conditions are right.

METHODOLOGY

To create healthy biomass, *Azolla microphylla* fronts were cultivated in polyhouses using 20X25x5cm plastic trays and Espinase and Watanabe (E and W) media. Harvested biomass was employed as an inoculant for subsequent tests after being cleaned with distilled water and gently blotted to remove excess moisture. Primary and secondary municipal wastewater that had undergone partial treatment was collected from the Nilothi sewage treatment plant in New Delhi. *Azolla* fronds (10g) were cultivated for seven days in trays. The growing media included tap water, E and W medium, main and secondary municipally treated water (in triplicates). In order to measure the natural attenuation, experiments were carried out using primary and secondary treated water that had not been inoculated as controls (due to the presence of natural micro flora in water).

By inoculating in primary and secondary treated sewage effluents, the growth properties of *Azolla* were investigated. The growth that was obtained in medium and tap water was compared to these. Following American Public Health Association standard methods for water and wastewater, initial and final levels of nutrients such as total and available phosphorous (P), total organic carbon (TOC), nitrite, and ammonia were examined. Accordingly, the ascorbic acid method, Wakley and Black method, nesslerization method for ammonia, and colorimetric method for nitrite were used to analyse total and available P, TOC, and nitrite, respectively. Physical characteristics were documented, such as pH.

RESULTS

In between 10-20 degree C maximum relative growth rate and minimum doubling time has been shown. In case of secondary treated effluent, a doubling time of 3.66 days was shown by *Azolla*. Around 35-40 degree C *Azolla* did not show any growth.

Water samples are collected to Check the parameters like total phosphorus, nitrate, ammonia etc. The brown colour of primary treated wastewater became colourless by the growth of *Azolla*. Growth of *Azolla* lowers the nitrate, phosphorus, ammonia levels. The biological oxygen demand is within limit in case of both the primary and secondary treated wastewater. The effluent from secondary treatment had an 80% reduction in total P value. Because phosphorus is the limiting ingredient for *Azolla*'s growth, the values for phosphorous have decreased. Ammonia levels in wastewater that had undergone secondary treatment had decreased by 54.8%. This demonstrates that ammonium ions did not always prevent *Azolla* from growing and fixing nitrogen (Kitoh et al, 1993). The wastewater's nitrite concentration was also examined. In

secondary treated wastewater, nitrite levels were discovered to be greater. Given the low ammonia level of the secondary treated wastewater and the nitrification process' conversion of ammonia to nitrite, this may be supported. After *Azolla* growth, the nitrite content was removed by 71.4%.

DISCUSSION

The potential of *Azolla* to perform wastewater bioremediation was examined, and the study's good results for removing total organic carbon, phosphorus, and nitrogen as well as its effectiveness in removing ammonia have been revealed. The presence of nitrogen and phosphorus causes algae to flourish (eutrophication), which depletes water bodies' oxygen supplies and kills native flora and wildlife. While the uninoculated controls kept had algal growth in them, the *Azolla* growths, which were discovered to be at their height at 20–35°C, did not allow the growth of algae. Algae grew in both main and secondary treated sewage water when the temperature was between 35 and 45°C, when *Azolla*'s growth and multiplication were minimal due to the unfavourable temperature. *Azolla* uses a low cost Espinase and Watanabe medium with no nitrogen added to it (Dawar and Singh, 2002; Yadav et al., 2014). This is due to the presence of *Anabaena Azollae* which is in association with *Azolla*. It fixes atmospheric nitrogen for *Azolla* (Pabby et al., 2003; Arora et al., 2003). *Azolla* could be used as a biofertilizer because of its higher nitrogen, phosphorus, potassium, and organic content, especially when it is produced in residential wastewaters and taken from wild habitats. Since *Azolla* can absorb phosphorus, its concentration in the plant was as high as that of the phosphate-rich growth media. If heavy metals are present, the utilized *Azolla* biomass can be used as green manure. If heavy metal is found, it should be burned in order to stop future environmental recycling or dried and removed for heavy metal extraction (Sood et al., 2012). Additionally, the potential of *Azolla* can be used to create functional built wetlands.

CONCLUSION

Azolla is a potential plant for preserving a sustainable environment since it improves soil quality, removes pollution, and has the extra benefit of dual cropping. It is a challenge for both policymakers and the scientific community to find ways to reduce the loss of ecosystem services, such as water, biomass, and global climate regulation. A promising organism for these issues may be *Azolla*. *Azolla* is therefore a perfect plant for polishing wastewater and is effective when utilized in artificial wetlands.

REFERENCES:

- i. Arora, A., Pabby, A., Singh, P, K. (2003). Heavy metal accumulation by *Azolla* in contaminated soils. Proc. 18th Ann. Intl. Conf. on Contaminated Soil Sediments and Water), Vol 8 Amherst Scientific Publishers, *Amherst Scientific Publishers Amherst, USA*. 8, 39-48

- ii. Arora, A., Saxena S. (2005). Cultivation of *Azolla microphylla* biomass on secondary-treated Delhi municipal effluents. *Biomass and Bioenergy*. 29, 60-64
- iii. Arora, A., Saxena, S., Shah, R. (2009). Aquatic microphyte *Azolla* for Nutrient Removal from Wastewaters in Constructed Wetlands. Proceedings of International Conference on Energy and Environment. Environmental Energy. 185-188
- iv. Arora, A., Singh, P, K. (2003). Comparison of biomass productivity and nitrogen fixing potential of *Azolla* spp. *Biomass and Bioenergy*. 24, 175-178.
- v. Bassi, N., Kumar, M, D., Sharmac, A, P. Saradhi,P. (2014). Status of wetlands in India: A review of extent, ecosystem benefits, threats and management strategies. *Journal of Hydrology*. 2, 1-19
- vi. Central Pollution Control Board. (2008). Status of water quality in India 2007, New Delhi, India: CPCB.
- vii. Dawar,S., Singh, P, K. (2002). Comparison of Soil- And Nutrient-Based Medium for Maintenance of *Azolla* Cultures. *Journal of Plant Nutrition*. 25(12), 2719-2729
- viii. DeBusk, T. A., Reddy, K, R. Hayes T, D., Schwegler, B, R. (1989). Performance of a pilot-scale water hyacinthbased secondary treatment system. *Journal WPCF*, 61, 1217–1224.
- ix. Elmachli, S., Chefetz, B., Vidal, L., Canals, A., Gedanken, A. (2010). Removal of silver and lead ions from water wastes using *Azolla filiculoides*, an aquatic plant, which adsorbs and reduces the ions into the corresponding metallic nanoparticles under microwave radiation in 5 min. *Water Air Soil Pollution*. 365-370
- x. Fisher, J. P. (1988). Wastewater treatment using aquatic plants. In (eds), *Alternative Waste Treatment Systems*, Rao Bhamidimarri, Elsevier Applied Science, Palmerston North, New Zealand: 34– 44.
- xi. Jaeger, E, C. (1978). *A Source-Book of Biological Names and Terms*, 3rd ed. Charles C Thomas, Publisher, Sringfield, Ill. 32
- xii. Kitoh, S., Shiomi, N., Uheda, E. (1993). The growth and nitrogen fixation of *Azolla filiculoides* Lam. in polluted water. *Aquatic botany*. 46 ,129-139
- xiii. Kollah, B., Patra¹, a, k., Mohanty, S, R. (2016). Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change. *Environ Sci Pollut Res*. 23:4358–4369
- xiv. Lumpkin, T, A., Plucknett, D, L. (1980). *Azolla*: Botany, physiology, and use as a green manure. 34 (2), 111-153
- xv. Millennium Ecosystem Assessment (MA), (2005). *Ecosystems and Human Well-being: Biodiversity Synthesis*. World Resources Institute, Washington, DC.
- xvi. Mujiyo, Sunarminto, B, H., Hanudin, E., Widada, J., Syamsiyah, J. (2016). Methane Emission on Organic Rice Experiment Using *Azolla*. *International Journal of Applied Environmental Sciences*. 11 (1), 295-308.
- xvii. Noorjahan C, M., Jamuna, S. (2015). Biodegradation of Sewage Wastewater Using *Azolla* Microphylla and Its Reuse for Aquaculture of Fish *Tilapia Mossambica*. *Journal of Environmental Science, Toxicology and Food Technology*. Vol. 9(3). 75-80
- xviii. Oron, G. (1990). Economic considerations in wastewater treatment with duckweed for effluent and nitrogen renovation. *Journal WPCF*, 62, 692–696.
- xix. Pabby, A., Prasanna, R., Singh, P, K. (2003). *Azolla anabaena* symbiosis from traditional agriculture to biotechnology. *Indian Journal of Biotechnology*. Vol.2, 26-37
- xx. Pandey, A., Verma, R, K., Mohan, J., Mohan, N. (2015). Utilization of *Azolla* aquatic plant as phytoremediation for treatment of effluent. *International Journal of Applied Research*. 1(2): 28-30
- xxi. Peters, G. A., Meeks, J. C. (1989). The *Azolla-Anabaena* symbiosis: basic biology. *Annual Review of Plant Physiology and Plant Molecular Biology* 40, 193-2 10

- xxii. Prasad, S, M., Singh, A. (2011). Metabolic responses of *Azolla pinnata* to cadmium stress: photosynthesis, antioxidative system and phytoremediation. *Journal of Chemistry and Ecology*. 27(6), 543-555
- xxiii. Ranalli, M., Lundholm, J, T. (2008). Biodiversity and Ecosystem function in constructed ecosystems. *CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources*. 3, 1-16.
- xxiv. Sachdeva, S., Sharma, A. (2012). *Azolla*: Role in Phytoremediation of Heavy Metals. Paper from Proceeding of the National Conference "Science in Media 2012" Organized by YMCA University of Science and Technology, Faridabad, Haryana (India)
- xxv. Singh, P, K. (1977). *Azolla* fern plant-rice fertilizer and chicken feed. *Kerala Karshakan*. 26, 5-6.
- xxvi. Singh, P, K. (1989). Use of *Azolla* in Asian Agriculture *Applied Agricultural Research*. 4(3), 149-161.
- xxvii. Singh, P, K., Panigrahi, B, C., Satpathy, K, B. (1981). Comparative efficiency of *Azolla*, blue green algae and other organic manures in relation to N and P availability in a flooded rice soil *Pl Soil*. 62, 35-44.
- xxviii. Sood, A., Uniyal, P, L., Prasanna, R., Ahluwalia, A, S. (2011). Phytoremediation potential of Aquatic Macrophyte, *Azolla*. *Ambio*, DOI 10.1007/s13280-011-0159-z
- xxix. Speelman, E, N., Van Kempen, M, M., Barke, J. *et al* (2009). The Eocene Arctic *Azolla* bloom: environmental conditions, productivity and carbon drawdown. *Geobiology*. 7(2), 155-170.
- xxx. Standard Methods for the Examination of Water and Wastewater. (1999). American Public Health Association (APHA), American Water Works Association, Water Environment Federation
- xxxi. Subudhi, B, P, R., Watanabe, I. (1981). Differential Phosphorus requirements of *Azolla* species and strains in phosphorus limited continuous culture. *Soil Sci.Plant Nut*. 27(2), 237-247
- xxxii. Vymazal, J. (2010). Constructed Wetlands for Wastewater Treatment. *Water* .2, 530-549
- xxxiii. Wagner, G, M. *Azolla*: A review of its biology and utilization. (1997). *Botanical Review*. 63, 1-26.
- xxxiv. Watanabe, I. (1982). *Azolla-Anabaena* symbiosis-its physiology and use in tropical agriculture. *In: Microbiology of tropical Soils and Plant Productivity*. 169-185.
- xxxv. Watanabe, I., Liu, C, C. (1992). Improving nitrogen-fixing systems and integrating them into sustainable rice farming. *P1. & Soil*. 141, 57-67.
- xxxvi. Yadav, R, K., Abraham, G., Singh, Y V., Singh P K. (2014). Advancements in the Utilization of *Azolla-Anabaena* System in Relation to Sustainable Agricultural Practices. *Proceedings of Indian National Science Academy* 80. 2, 301-316
- xxxvii. Soman,D;Anitha,V;Arora,A(2018) Bioremediation of municipal sewage water with azolla microphylla