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Determination of the ability of *Azolla* as an agent of bioremediation

Maibam K. Devi[1], Wahengbam N. Singh[2], Wahengbam R. C. Singh[1], Heigrujam B. Singh[1*] and Nongmaithem M. Singh[3]

[1]DBT- Institutional Biotech Hub, Deptt. of Biotechnology, S. K. Women's College, Nambol, Manipur
[2]DBT- Institutional Biotech Hub, Kamakhya Pemton College, Hiyangthang, Manipur
[3]DBT- Institutional Biotech Hub, Pettigrew College, Ukhrul, Manipur

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ABSTRACT

Azolla is a good bioremediation and can be used for the treatment of wastewater such as industrial effluents, sewage water etc. and it is eco-friendly and effective. We investigated the ability of the aquatic fern Azolla to determined the pH, Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD) of the water sample which is collected from Nambul River and Imphal River. The collected water sample is stored in a tub and 1m^2 microplots is prepared with sufficient water (5 -10 cm) , 4-20 kg P_2O_5/ha, pH (8.0) , temperature (14 - 30^0 C). The fresh Azolla (0.5 – 0.4 Kg/m^2) is inoculated in the microplots using furadon as an insecticide. After three weeks of growth, mat formed by Azolla is harvested and the same microplot is inoculated with fresh Azolla to repeat the cultivation.The pH, DO and BOD of the water was taken before and after treatment of the Azolla. The pH of the water sample has come down after the treatment and brought more towards neutral this may due to absorption of the ions and other organic pollutants present in the water sample by Azolla. The D.O. of the water was also found to be increased and the level of BOD came down significantly.

Keywords: *Azolla*, Bioremediation, wastewaters, pH, D.O. and B.O.D.

INTRODUCTION

The area of polluted soil and water is expanding day by day due to rapid increase in world population. Environmental pollution is increasing due to increased urbanization and industrialization. The toxic wastes originating from industrial units, automobile engines and domestic operators are basically disposed into the components of our environment such as soil, air and water. Accumulation of such toxic water basically into the soil and water ultimately enter the food chain and cause health hazards. These toxic substances are either organic or inorganic compounds.

Agricultural operations like application of chemical fertilizers, herbicides, insecticide, nematicides etc., have resulted into the addition of organic pollutants. Whereas the inorganic compounds that pollute the environment are heavy metals, metalloids, trace elements and radioactive materials which are added through different human factors and other geophysical modifications.

Applications or remediation approaches become imperative when the buildup of these toxic substances in water and soil is beyond permissible limits. Novel Biotechnology approaches are being applied for treating wastes and these include bioremediation and phytoremediation. These are the terms used to describe those methodologies which employ living organisms to remove toxic compounds from the environment. Bioremediation refers to the use of lower organisms (viz., algae, bacteria and fungi) to reduce or eliminate toxic pollutants from contaminated sites by degradation, assimilation or transpiration in the atmosphere. It is the technology for removing pollutants from the environment, restoring contaminated sites and preventing future pollution. Bioremediation of organic contaminants is primarily based on either microorganism naturally present at the site, or on microbial inoculants developed in the laboratory and introduced at the site. Certain bacterial, fungal and algal species are capable of accumulating some toxic inorganic contaminants as well.

Water Quality
Water quality is determined as a set of chemical, physical and biological features of the water, which determines the suitability of water for various uses Water analysis is done to achieve the following objectives:

- To safeguard human health
- To control the pollution level of water bodies.
- To maintain and improve the quality of water for various uses like amenity, fisheries, irrigation etc.
- To protect and conserve aquatic flora and fauna.

A number of quality parameters are measured to determine water quality. These parameters include physical properties like pH, color, turbidity, suspended solids, temperature, conductivity, odor etc. Chemical properties such as COD (Chemical Oxygen Demand), BOD (Biological Oxygen Demand), DO (Dissolve Oxygen), total nitrogen, total phosphate, total pesticides etc, and Biological properties e.g., total coliform bacterial, faecal coliform counts, faecal streptococci counts, Salmonella counts etc.

Water Quality Issues
Contamination can enter the water bodies through one or more of the following ways:

Direct point sources: Transfer of pollutants from municipal industrial liquid waste disposal sites and from municipal and household hazardous waste and refuse disposal sites.

Diffuse agricultural sources: Wash off and soil erosion from agricultural lands carrying materials applied during agricultural use, mainly fertilizers, herbicides and pesticides.

Diffuse urban sources: Run off from city streets, from horticultural, gardening and commercial activities in the urban environment and from industrial sites and storage areas.

Water quality issues needing to be addressed with respect to river is presented below.

Rivers:
Change in Physical Characteristics
Temperature, turbidity and total suspended solids (TSS) in rivers can be greatly affected by human activities such as agriculture, deforestration and the use of water for cooling. For example, the upward trend in soil erosion and the related increase in TSS in rivers can be seen in most of the mountainous regions in India.

Contamination by Faecal and Organic Matter
In India faecal contamination is still the primary water issue in rivers, especially where human and animal wastes are not adequately collected and treated. Although this applies to both rural and urban areas, the situation is probably more critical in fast –growing cities.

The release of untreated domestic or industrial wastes high in organic matter into rivers results in the marked declined in oxygen concentration (sometimes resulting in aerobic conditions) and a rise in ammonia and nitrogen concentrations, downstream of the effluent input. The most obvious effect of the release of organic matter along the length of the river is the depletion of oxygen downstream of the discharge as shown by the so- called 'oxygen-sag curve' which plots dissolve oxygen concentration against distance. Industrial activities which discharge large organic loads include , pulp and paper production and food processing. Faecal matter affects the use of water for drinking water source or bathing water, as well as ecological health of river.

Toxic Pollutants: Organic and Heavy Metals
Organic pollutants (Mostly chemicals manufactured artificially by man) are also becoming an important water quality issue. They enter rivers as:
- point sources directly from sewers and effluent discharges (Domestic, Urban and industrial sources)
- diffuse sources from the leaching of solid and liquid waste dumps or agricultural land run off.
- indirectly through long-range atmospheric transport and deposition.

Uncontrolled discharge of industrial wastewater often causes pollution due to toxic metals. Other sources of metal pollution are leachates from urban solid waste landfills and mining waste dumps.

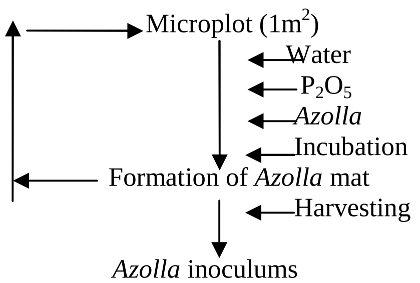
Health impacts of water pollution:
It is a well-known fact that clean water is absolutely essential for healthy living. Adequate supply of fresh and clean drinking water is a basic need for all human beings on the earth, yet it has been observed that millions of people worldwide are deprived of this.

Freshwater resources all over the world are threatened not only by over exploitation and poor management but also by ecological degradation. The main source of fresh water pollution can be attributed to discharge of untreated waste, dumping of industrial growth, urbanization and the increasing use of synthetic organic substances have serious and adverse impacts on freshwater bodies. It is a generally accepted fact that the developed countries suffers from problems of chemical discharge into the water sources mainly groundwater, while developing countries face problems of agricultural run-off in water sources. Polluted water like chemicals in drinking water causes problem to health and leads to water-borne diseases which can be prevented by taking measures which can be taken even at the household level.

MATERIALS AND METHODS

Collection of water sample:
The water sample is collected from Nambul River,Imphal River and are stored in tubs. An approximate amount of 10 litres per tub is used. The water sample is treated with fresh *Azolla* in the tub itself, while one of the tub is used for the control experiment. All the parameters are checked simultaneously for the test as well as the control experiment. The *Azolla* use for treatment is collected fresh from the stock culture maintained in microplots.

Culture of *Azolla:*
Microplots (1m^2) are prepared in nurseries in which sufficient water (5 -10 cm) is added. For good growth of *Azolla*, 4-20 kg (Phosphorous pentoxide) P_2O_5/h is also amended. Optimum pH (8.0) and temperature (14 - 30^0 C) is maintained. Finally, microplots are inoculated with fresh *Azolla* (0.5 – 0.4 Kg/m^2). An insecticide (furadon) is used to check the attack of insects. After three weeks of growth, mat formed by *Azolla* is harvested and the same microplot is inoculated with fresh *Azolla* to repeat the cultivation.



Determination of pH:
The pH of the water sample is checked by using a pH Meter. The pH of the water sample is checked on the first day before treatment with *Azolla* and then on the 14th day after treatment with *Azolla* and the change in pH (if any) is recorded.

Determination of Dissolved Oxygen:
Dissolved oxygen of water is of paramount importance to all living organisms and is considered to be the lone factor which to a great extent can reveal the nature of the whole aquatic system at a glance, even when information on other chemical, physical and biological parameters is not available.

The estimation of DO is done by the titrimertic method. The oxygen of the water combines with manganese hydroxide, which on acidification liberates iodine equivalent to that of oxygen fixed. This iodine is titrated by standard sodium thiosulfate solution using starch as indicator.

$$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K_2SO_4$$
$$Mn(OH)_2 + O \longrightarrow MnO(OH)_2$$
$$MnO(OH)_2 + 2H_2SO_4 + 2KI \longrightarrow MnSO_4 + K_2SO_4 + 3H_2O + I_2$$

Procedure:
- Collect water sample without bubbling in the 250ml glass bottle.
- Add 2ml each of manganous sulfate and alkaline iodide-azide solutions in succession, right at the bottom of the bottle with separate pipettes and replace the stopper.
- Hake the bottle in the upside down direction at least six times.
- Allow the brown precipitates to settle
- Add 2ml of conc. Sulfuric acid and shake the stopper bottle to dissolve the brown precipitate.
- Take 50ml of sample in a flask and titrate with thiosulfate solution (taken in the burette) till the color changes to pale straw.
- Add 2 drops of starch solution to the above flask which changes the color of the contents from pale to blue.
- Titrate again with thiosulfate solution till the blue color disappears.

Calculation of D.O:
The total amount of sodium thiosulfate solution (titrant) used found out and the dissolved oxygen content of water (mg/L) is calculated by applying the equation:

$$\text{D.O. (in mg/L)} = \frac{\{*8 \times 1000 \times N\}}{V} \times v$$

Where,
V = Volume of sample taken (ml)
v = volume of the titrant used
N = Normality of the titrant

*8 is a constant since 1ml of 0.025 sodium thiosulfate solution is equivalent to 0.2 mg oxygen.

In the same manner as in pH, the Dissolved Oxygen is checked on the first day prior to treatment with *Azolla* and then on the 14^{th} day of *Azolla* treatment. The difference in the D.O. is also recorded.

Determination of B.O.D:
The Biochemical Oxygen Demand is a way of expressing the amount of organic compounds in sewage as measured by the volume of oxygen required by bacteria to metabolize it under aerobic conditions. It is a good index of the organic pollution. If the amount of organic matter in sewage is more, the more oxygen will be utilized by bacteria to degrade it. Dumping sewage that contains high B.O.D. increases the concentration of soluble organic compounds in the aquatic body where it is discharged. Digestion of these organic compounds in natural eco systems such as lakes, rivers can deplete available oxygen and result in asphyxiation (death) of fish.

The B.O.D. of a water sample is generally measured by incubating the sample at 20^0 C for 5 days in the dark under aerobic conditions (B.O.D. 20^0C). Since nitrification consumes oxygen significantly, thereby resulting in overestimation of B.O.D. and must be checked by adding 1ml of 0.5% solution of allylthiourea. In the water sample where more than 70% of the initial oxygen is consumed, it is necessary to aerate/oxygenate and /or dilute the sample with B.O.D. free water to avoid oxygen stress.

Procedure:
- Adjust the pH of the water sample to neutrality using 1N acid or 1N Alkali solution.
- Fill the water sample in 6BOD bottles without bubbling.
- Add 1ml of allylthiourea to each bottle.
- Determine dissolved Oxygen content in three of the 6 BOD bottles by the titration method.
- Take the mean of the three readings.(D_1)
- Incubate the rest of the three BOD bottles at 27^0C in a BOD incubator for 3 days.
- Estimate the Oxygen concentration in all the three incubated samples.
- Take the mean of the three readings (D_2)

Calculation of B.O.D:
The BOD of the water is calculated in mg/L by applying the formula BOD_3 in mg/L = D_1-D_2
Where,D_1 = Initial DO in sample (mg/L)
D_2 = DO after 3 days incubation (mg/L)

Like in pH and D.O., the B.O.D is also checked before and after treatment of *Azolla* on the water sample and the change in B.O.D is recorded.

RESULTS AND DISCUSSION

1. pH: The pH of the water before treatment of the water sample was found to be 7.89. But after treatment of the sample with *Azolla* for two weeks it was found that the pH of the water sample has come down to 7.4.
2. Dissolved oxygen (DO): The Dissolved Oxygen of the water sample before treatment with *Azolla* was found to be 10.9mg/L and after treatment with *Azolla* for two weeks, it was found that the D.O. has increased to 14.2 mg/L.
3. Biochemical Oxygen Demand (BOD): The B.O.D. of the water sample prior to treatment with *Azolla* was found to be 3.3mg/L and after treatment with *Azolla* for 14 days, it came down to 2.8 mg/L.

Fig: Results table for before and after *Azolla* treatment of the three parameters pH, DO and BOD.

Parameters	Before *Azolla* Treatment	After *Azolla* Treatment for 14 days
pH	7.89	7.4
Dissolved Oxygen	10.9 mg/L	14.2 mg/L
Biochemical Oxygen Demand	3.3mg/L	2.8 mg/L

In this three parameters i.e., pH, Dissolved Oxygen and Biochemical Oxygen Demand, have been studied.

The pH of the water is brought more towards neutral, i.e., it has become more portable as a result of *Azolla* treatment. This may be a result of absorption of the ions and other organic pollutants present in the water sample by *Azolla*. The increased in the D.O. can be explained as the *Azolla*, being an aquatic plant produces oxygen by the process of Photosynthesis. Consequently the amount of the dissolved oxygen is increased. The BOD of the waste water is also improved as a result of *Azolla* treatment. This gives the conclusion that *Azolla* can be used as a good and reliable agent of Bioremediation which any layman can easily understand.

CONCLUSION

From the above observation it can be concluded that *Azolla* is a good bioremediation and can be used for the treatment of wastewater such as industrial effluents, sewage water etc. on a large scale.

The wastewater treatment processes employing *Azolla* are advantageous over chemical methods, as they are eco-friendly and effective.

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