Scavenging Efficiency of Azolla pinnata in effluent as Remediation Agent

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Abstract - *Sugar industry being an important role in the Indian economy and also plays a very vital part in polluting the environment with its waste discharge. The study focused to find out whether algae could be used as a prime source to degrade the effluent. Azolla pinnata showed significant growth in the diluted cane sugar industry effluent and analyzed the composition of their effluents which are the primary source of water pollution. The results revealed polluting constituents level might be reduced with reference to parameters such as BOD, COD elements, total suspended solids. The relative growth of Azolla pinnata in the diluted effluent was positive and growth rate declined in the untreated effluent which reflected utilizing capacity of dissolved and suspended solids had potential to enhanced growth of Algae by degraded mechanism thereby it reduce the toxicity. The algae grown culture filtrate used for Oryza sativa seed germination and bioassay studies which resulted self sufficient nitrogen fixing system was investigated for detoxification. There was an increased accumulation of biochemical contents in the cells grown in the diluted effluent. Green algae can be used as remediating agent for effluent also recommended as inoculate algae to grow for a period before disposal. Hence, from the investigation it might be concluded that algae showed signified growth in diluted effluent through the analysis phytoremediation techniques suggested for industrial effluent treatment. Sugar cane industry effluent after treated with Azolla was found to be suitable for irrigation purpose also Algae biomass can be consider for fertilizer use. Azolla, biomass, chlorophyll content of RGR were enriched due to the presence of minerals and other dissolved materials present on the 25% of diluted effluent utilized for its growth but the growth was were reduced at 50%, 75% (volume by volume in water) and 100%.Killed the aquatic plant Azolla which was reported on the day of inoculated itself due to inhibition on biochemical process thereby decay reported.*

Key Words: Azolla pinnata, Chlorophyll, Effluent, Phytoremediation, Pollution

1. INTRODUCTION

The environmental pollution is one of the most severe problems nowadays. Moreover, accumulated pollutants may have adverse effects to living organisms leading eventually to ecosystem degradation. Living organisms are possessing natural abilities of either the removal of pollutants or their degradation from organism substrate. This technique is called biotic remediation and it is broadly used where there is a need for water purification and soil reclamation [1]. Sugar industry is one of the most important agro based industry segment in India. Cane Sugar Industry being an important role in the Indian economy as well as in the foreign exchange earnings and also plays a very vital part in polluting the environment with its waste discharge. With expansion of Sugar plants, pollution due to inadequate and it becomes threat for environment.

In the sugar industry, water is used for cleaning purposes in the different sections of the factory generates wastewater. Practically, there are no single units which generate wastewater but the wastewater is produced mainly by washing on the milling house floor, boiling house like evaporators, clarifiers, vacuum pans, centrifugation, etc. Additionally, periodical cleaning of lime water and SO_2 producing house is also contributing to the huge volume of wastewater, as well as periodic decaling of heat exchangers and evaporators using NaOH, Na_2CO_3 and HCl for neutralization [2]. Sugar cane molasses, also referred to as the final effluent of sugar refinement is a dense, darkly colored substance teeming in minerals. In addition, molasses has been widely advertised for its therapeutic properties believed to be a result of its rich mineral content. However, little or no scientific evidence exists to corroborate the suggested health benefits of this substance. Traditionally, molasses has been used as an alternate sweetener to sugar and included as a common ingredient in various food products. Worldwide, molasses is primarily used as feed for livestock as it enhances microbial growth in the rumen of animals that promotes the digestion of fiber and non-protein nitrogen.

Studies of physicochemical properties of the sugar industrial effluent which were collected and analyzed from the mill house, process house and final combined wastewater indicated that the effluent qualities and quantities are quite different [3]. Sugar industry effluent quality varies depending on chemical types utilized, the nature of the sugarcane such as the cane variety and the nature (physicochemical and biological) of soil in which the cane is grown. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides [4]. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, bio-fertilizers do not contain any chemicals which are harmful to the living soil.

The *Azolla* group contains only 6 or 7 species which are the smallest ferns in the world. *Azolla pinnata* spreads rapidly by vegetative growth and can form dense mats and forms extensive colonies on still or slow moving freshwater bodies such as ponds, lakes, ditches and canals. It thrives in eutrophic conditions could block the interface between the water surface and the atmosphere. *Azolla pinnata* has a tremendous potential to take up heavy metals among which include Mercury and Cadmium (70–94%) may be used as a bio-accumulator to absorb heavy metals in effluent [5]. *Azolla* is used as a food supplement fresh dried or ensiled for a variety of animals, including pigs, rabbits, chickens, ducks, and fish [6]). *Azolla* fed to broilers resulted in growth and body weight values similar to those resulting from the use of mustard oil cake meal [7]. 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 as a process of bioremediation.

In the present study has focused for the analysis of the composition of sugar industry effluent and evaluates the alternative treatment options used to remediate on the challenges impacts of effluent through *Azolla pinnata* can be considered as phytoremediation approaches.

2. MATERIALS AND METHODS

The sugar cane industry effluent was collected from Rajashree sugar cane industry near Theni Dt, Tamil Nadu, India. The effluent was analyzed following the methods outlined by APHA [8] before and after introduction of the *Azolla* plants in the effluent water samples.

Plant materials used in this study was *Azolla pinnata* collected from *Azolla* farm and water pond which located in Theni Dt, Tamilnadu, India, the samples were collected in polythene containers (5 liters). The plants were washed thoroughly and maintained in tap water for 15 days to acclimatize to laboratory conditions.

The physico-chemical parameters of effluent such as color, pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and minerals Ca, Mn, Zn, K, Na, Mg, Fe, Pb, Cu, and Chromium were determined by Atomic Absorption Spectrophotometry (Perkin –Elmer, model 500)

3. EXPERIMENTAL DESIGN

Batch experiments were conducted in 4 plastic tubs (5litres capacity). 5 to 10 g of sample *Azolla pinnata* were introduced in the effluent made of dilution in various ranges, 25, 50, 75 and 100% up to 3 liters /tub which were kept separately for a period of 30 days. The inoculated *Azolla pinnata* samples were collected and used for further experiments.

3.1 Estimation

Total chlorophyll was estimated according to the method of Arnon [9]. Relative Growth Rate (RGR) was determined by using the formula suggested by Williams [10]. Seedling bioassay was carried out by the method of koptiyeva and Tantsiurenko [11] with modification of Renuga [12] .*Oryza sativa* variety IR-50 seeds were placed in Petri dishes kept moist by layers of cotton and a course filter paper. Thirty seeds per Petri dishes of *Oryza sativa* were used five replicates were maintained for each of the concentration. A portion of (20 ml) of each concentration of the effluent inoculated with *Azolla pinnata* was added to Petri plate on alternate days and the controls received the same amount of distilled water. The germination percentage was calculated 5 days after germination started then 10 germinated seeds were selected from Petri plate transfer to beaker provide with same concentration after that allow it for 5 days to grow and measured root and shoot length separately in all three replicates in each of the treatments were employed.

3.2 Estimation and Discussion

In the present study growth of the *Azolla pinnata* are independence external source of minerals allows the fern to double its biomass every two to three days at room temperature and the amount of biomass has been used as a green fertilizer in rice field in India.

Fig -1: Growth of *Azolla pinnata* culture

Legend: A. *pinnata* is a small fern with a triangular frond measuring up to 2.5 centimeters in length which floats on the water. The frond is made up of many rounded or angular overlapping leaves are green in color and given a velvety appearance and the water body might be coated in a dense layer of the plants, which form a velvety mat that crowds out other plants.

Azolla is floating freshwater ferns absorbed pollutants and heavy metals and the accumulates were analyzed to order characterize the efficiency of plant make it a perfect candidate for bioremediation systems, its biomass is easy to harvest and desiccates very fast (Cohen et al., 2004), although it is always important to assess the potential impact of introducing *Azolla* lives in waste water to be used as absorbing agent of minerals which can utilize the content for its growth thereby doubling process confirmed (Fig1).

Physicochemical characteristics of the effluent revealed color of the waste water was recorded as dark brownish due to the presence of sugar residues in the waste water. The pH of the effluents is generally depends on process involved in of industry. Final effluent samples showed more acidic in nature, since it contained the acidic pH 4 the values of pH are due to the change in the manufacturing process of industry and several chemicals are used for coagulation of impurities.

Results given in the Table1 revealed effluent created warm condition at the point of discharged area in water bodies. The reported waste water temperature was changed from 70^{o}C to 37^0 C due to thermal stratification which showed water temperature fluctuations.

Table -1: Characteristics of Sugarcane industrial effluent

S.No	Parameters	Before Inoculation (mg/L)	After Inoculation(mg/L)
1	Color	Thick Black	Normal
2	Odour	Bad	Normal
3	pH	4	6.5
4	Temperature	70℃	37℃
5	BOD	49000	8590
6	COD	24500	7490
7	Total Solids	950	758
8	Suspended solids	95	78.5
9	Dissolved Solids	2689	987
10	Volatile Solids	47000	600

Valves are means of three replicates

The suspended particle absorbed heat efficiently also supported to increase biomass of plants. Sugarcane molasses spent wash after biological treatment results in significant removal of BOD and COD from the effluent. Oxidation process could achieve 90% decolorization for biologically treated spent wash with simultaneous COD reduction. It also resulted in improved biodegradability of the effluent.

The RGR of *Azolla pinnata* was found to increase steadily with increasing in diluted concentration of effluent along with its growth duration [Figure2]. RGR is a good measure of stress induced changes and sharp decrease in RGR indicates severe limitation on based on available resources which reflect the overall growth of plants might be of different.

The positive growth has been observed in different concentration of effluent inoculated with *Azolla pinnata* in which highest growth rate at 25% when compared with control and other concentration of effluent. Figure 2 explained relative growth rate of *Azolla pinnata* enhanced in diluted in industrial effluent due to uptake of nutrient contents from dissolved solids. The results obtained in aquatic plants can be consider as positive factor to stimulate higher plant growth, gardeners and farmers use as biofertilizer for agriculture field.

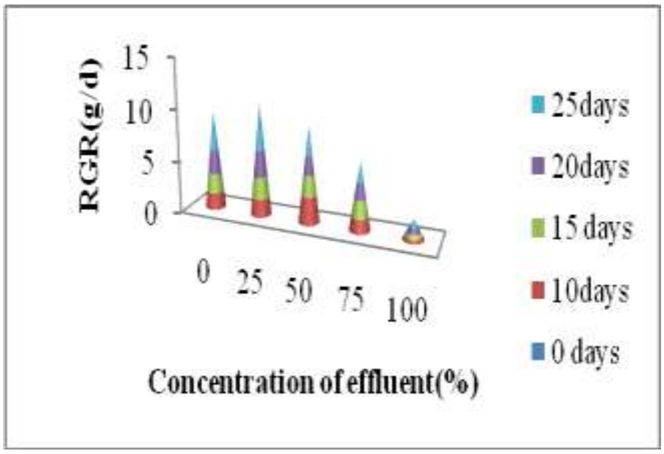

Fig -2: Determination of Relative growth rate of Azolla pinnata in diluted effluent

Legend: Fig 2 indicate growth rate of *Azolla pinnata* species were estimated based on dry weight of the sample harvested after the time intervals mention in materials and methods. Each valve means of three replicates.

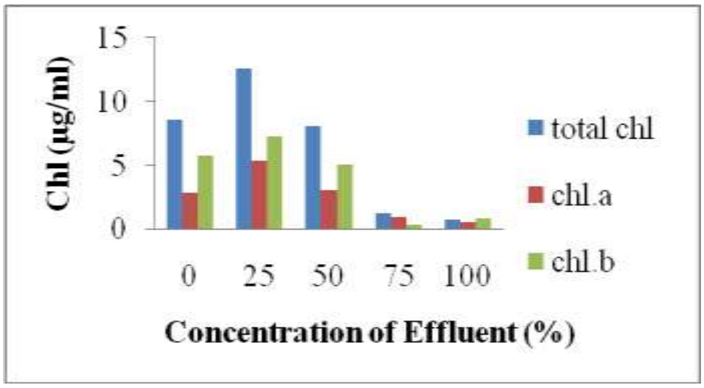

Fig -3: Estimation of Chlorophyll contents in *Azolla pinnata* after grown in effluent

Legends: X axis indicates various dilutions of the effluents; Y axis indicates chlorophyll content of *Azolla pinnate* grown in effluent; Valves are means of three replicates.

As bioremediation techniques any type of might be utilized as irrigation purpose after treated with algae in the presence of highly diluted effluent which has essential macronutrients which could act as growth promoting factor for higher plants. Most fertilizer on the market contains large amounts of potassium, phosphorus and nitrogen. Since the other chemical/ elements found in the effluent are obtained by aquatic plants used as secondary nutrients that plants need such as Mg, Ca, Cu, Mn, Zn Na, Fe without these micronutrients plant would not thrive other three macronutrients N, P, K for higher plant growth.

The chlorophyll content increased at 25% tannery effluent treatment [Fig 3] suggesting that the synthesis of chlorophyll is accelerated at low concentration of the effluent. Photosynthetic pigments synthesis enhanced due to optimal uptake of minerals /elements from effluent in the experimental set up mention in methods. Figure 3 revealed increased chlorophyll contents as increased in dilution of the effluents subsequently decreased contents of chlorophyll at higher concentration of effluents.

Table -2: Analysis of minerals in the effluent

Parameters	Concentration(mg/lit)
Potassium	386.0
Copper	0.9
Magnesium	679.0
Sodium	487.0
Zinc	1.6
Phosphorus	432
Calcium	541.0
Lead	0.2
Manganese	268.0
Nitrogen	312

Table 2 shows the concentration of minerals profile of higher in order Mg > Ca > Na > P > K > N > Mn were detected and Zn > Cu > Pb concentrations were low.

Microscopic phytoplankton play some of the biggest roles in climate control, oxygen supply and food production. Algae are responsible for more than 40% of Earth's photosynthetic production that process uses up carbon dioxide, which helps regulate CO_2 levels in the atmosphere, and produces oxygen for other organisms to live.

Algae growth rely on photosynthesis to produce energy still need other nutrient to grow and reproduce, these nutrients are phosphorus, nitrogen are present in water body caused eutrophication which is an indicator algae bloom detrimental to water quality and aquatic health. *Azolla pinnata* was observed to purify waters polluted by two heavy metals Hg and Cd under a microcosm condition.(Rai,2008).The free floating aquatic fern, also has distinct advantages for use in remediation treatments because it is simple and cheap to grow, it has high biomass productivity coupled with a high rate of N fixation, the ability to grow in varied environments and concentrate nutrients, and it can be used in multiple applications as biofertilizer, animal feed, biofilter, bioweedicide, and heavy metal phytoremediation from floodwater.

Results reported that there was a gradual decrease in the percentage seed germination and germination value with sugar industry effluent concentration. The untreated sugar industry effluent could possibly lead to soil deterioration and low productivity. Terrestrial and aquatic environmental pollution could be averted by proper treatment of the effluents using suitable conventional methods. In conclusion, sugar industry effluent concentration governs seed germination. The effects vary from crop to crop because each plant species has its own tolerance of the different effluent concentrations.

Azolla removes nitrogen and phosphorus, elements that cause water eutrophication also has been extensively used in Asian countries for N fertilization of paddy crops and as green manure useful in treating industrial waste water, which may be used for irrigation, and the biomass produced can be used as biofertilizer or green manure after mild treatment.

Fig -4: Effect of effluent after treated with *Azolla pinnata*

Legends: Growth of *Oryza sativa* in the culture filtrate of *Azolla pinnata* inoculated in various concentration of effluent given in fig 4 indicated 1 – Control ; 2- 25%; 3-50%, 4-75%

Bio fertilizer is a substance which contains living organisms which promotes growth by increasing the supply or availability of primary nutrients to the host plant. Aquatic macrophytes in natural and constructed wetlands proved to be a potent tool for the treatment of heavy metals from industrial effluents. Physico-chemical factors like temperature, pH, light, salinity, and presence of other heavy metal may affect the metal uptake. Both live and dead biomass of macrophytes may be used in phytoremediation, though dead biomass is generally preferred in the treatment of industrial effluents due to reduced cost, easy disposal, and lack of active biochemical machinery leading to metal toxicity and death of plants. Biomass disposal problem and seasonal growth of aquatic macrophytes are some of the limitations in the transfer of phyto-remediation technology from the lab to the field. However, an eco-sustainable model has been developed through our various works that may curb some of the limitations.

Disposed biomass of macrophytes may be used for many fruitful applications. Bio-fertilizers are cost-effective relative to chemical fertilizers which has lower manufacturing costs, especially regarding nitrogen and phosphorus use. Water pollution might be treated by growing various algae species, *Azolla* is a small, eukaryotic, aquatic fern having global distribution belonging to Prokaryotic blue green algae is an alternative nitrogen source. This association has gained wide interest because of its potential use as an alternative to chemical fertilizers. *Azolla* may also be used to accumulate macro-and microelements from sewage effluent and polluted water.

3. CONCLUSIONS

Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, bio-fertilizers do not contain any chemicals which are harmful to the living soil. Macrophytes are potent tools in the abatement of heavy metal pollution in aquatic ecosystems receiving industrial effluents and municipal wastewater which are preferred over other bio-agents due to low cost, frequent abundance in aquatic ecosystems, and easy handling.

Thus the present study highlighted the possible usage of effluent as growth adjuvant at a diluted concentration of 25% when the availability of water required for dilution is not limiting. Results were recommends that cultivation of *Azolla pinnata* facilitates wastewater management through recycling and reuse of municipal wastewater and is a fruitful strategy for sustainable and phytoremediation capacity of a small water fern, *Azolla pinnata* was observed to purify waters polluted by industrial waste disposal region in India.

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