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Bioremediation of textile effluent using *Aspergillus niger* Van Tieghem.

Authors:
Sheela C, Josmin Laali Nisha LL** and Poonguzhali TV***

Institution:
*Assistant Professor, P.G and Research Department of Botany, Queen Mary's College-600 004.

**Research scholar, Department of Botany, Queen Mary's College, Chennai-600 004.

P.G and research department of Botany, Queeen Mary's College, Chennai-600 004, India.

ABSTRACT:

Bioremediation is the most promising and less expensive way for cleaning up pollution contaminated soil and water. Mycoremediation utilises, mainly microorganisms, e.g. yeast or fungi to clean up contaminated soil and water. The contamination of toxic chemicals and heavy metals in the environment is an ever increasing and serious issue which threaten humans, animals, and the present ecosystem. Textile waste water was collected from Guindy (SIDCO Industrial Estate) and its physico-chemical parameters was analysed based on APHA. *Aspergillus niger* Van Tieghem was the test organism, collected from the culture collection centre, Centre for Advanced Studies in Botany, University of Madras. Fungal culture was maintained in Murashige and Skoog medium and further experiments were conducted in these media and amendments. *Aspergillus niger* was grown in different concentrations of the effluent. Mycoremediated effluent were used as foliar sprays (sprayed twice of 100 ppm concentration) on *Solanum nigrum* Linn.. After 30 days parameters such as height of the plant, length of the leaf and chlorophyll, carbohydrates, protein and lipid were determined.

Keywords:
Mycoremediation, *Aspergillus niger, Solanum nigrum*, physioco-chemical, morphological and Bio-chemical parameters.

Corresponding author:
Poonguzhali TV.

Email:
pooqmc@gamil.com
josminll@rediffmail.com.

Phone No:
+91-9841380698

Web Address:
http://www.ecologyresearch.info documents/EC0011.pdf.

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INTRODUCTION

Groundwater is one of the most vital sources of water on earth for all the living beings in this world. Bioremediation is the best option to destroy harmful pollutants using natural biological activity. It is relatively low-cost which generally have a greater public acceptance and can often be carried out on contaminated site (Vidali, 2001).

Bioremediation activity using microbe is stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates (methane, phenol, andtoluene), or by introducing microorganisms with desired catalytic and metabolic capabilities (Ma *et al.,* 2007; Baldwin *et al.,* 2008). Soil microbes develop a rhizospheric zone or root nodules (highly complex symbiotic and synergistic relationships) which acts as a tool for accelerating the rate of degradation or to remove contaminants.

Compared to other methods, bioremediation is the most promising and less expensive way for cleaning up pollution contaminated soil and water (Kamaludeen *et al.,* 2003). Most important parameters for bioremediation are i) the nature of pollutants, ii) the soil structure, pH, Moisture contents and hydrogeology, iii) the nutritional state, microbial diversity of the site and iv)Temperature and oxidation-reduction (redox- Potential) (Dua *et al.,* 2002). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Tang *et al.,* 2007).

Mycoremediation uses, mainly microorganisms, e.g. yeast or fungi bacteria to clean up contaminated soil and water (Strong and Burgess, 2008). The contamination of toxic chemicals and heavy metals in the environment is an ever increasing and serious issue which threaten humans, animals, and the present ecosystem. Mycoremediation practices involve mixing of mycelium (the vegetative part of a fungus) into contaminated soil, placing mycelial mats over toxic sites, or a combination of these techniques, in one or more treatments. ("Mycelium Running -How Mushrooms Can Save the World").

The use of fungi to control or remediate polluted soils, is an emerging technology, which throws the light for tomorrow's pollution contaminant free earth. Organic farming is based on holistic, ecologically balanced agricultural principles involving soil fertility, crop rotation and natural pest control. It may sound like an elusive concept, but the basis for organic farming is actually very simple: Allow nature to do what nature does best.

Organic farming paves way for the development of microbiological soil life. Plants are naturally supplied with a whole range of nutrients that would otherwise be too distant, insufficiently supplied or physically unavailable for uptake by the plant roots alone. So in the organic system, the biological activity within the soil is fundamental to the breakdown of organic matter and the delivery of the range and quantity of nutrients required by the crop (Balfour, 1943).

MATERIALS AND METHODS

Collection of effluent

Water sample from the textile effluent was collected from SIDCO Industrial Estate, Guindy during September 2010 at around 10.00 AM. Samples were collected in black polythene containers for detailed physico-chemical analysis. (APHA, 1985).

Growth of Fungi in the laboratory condition

Aspergillus niger Van Tieghem was obtained from the culture collection centre, Centre for Advanced Studies in Botany, University of Madras. Fungal culture was maintained in Murashige and Skoog medium (Murashige and Skoog, 1962) and further experiments were conducted in these media and amendments. Growth of *Aspergillus niger* in different concentrations of the effluents was studied by fresh weight/dry weight method.

Mycoremediation experiments

The textile effluent was treated with the micro fungi *Aspergillus niger* diluted with five different

Table 1 Physico-chemical characteristics of textile effluent from Gundy (SIDCO Industrial Estate)

S.No.	Physical characteristics	Date and collection 21. 09. 2011 Textile effluent
1	Appearance	Turbid
2	Temperature	27°C
3	Odour	Offensive smell
4	Hydrogen-ion concentration (pH)	7.41
5	Turbidity NTU	85.3
6	Total solids (TS) mg/L	1672
7	Total suspended solids (TSS) mg/L	32
8	Total dissolved solids (TDS) mg/L	1640
9	Electrical conductivity (micro mho/cm)	2327
10	Alkalinity ph (as $CaCo_3$) mg/L	0
11	Alkalinity total (as $CaCo_3$) mg/L	360
12	Total hardness (as $CaCo_3$) mg/L	350
13	Calcium (as Ca) mg/L	84
14	Magnesium (as Mg) mg/L	34
15	Sodium (as Na) mg/L	370
16	Potassium (as K)	25
17	Manganese (as Mn) mg/L	Nil
18	Free Ammonia (as NH3) mg/L	29.12
18	Nitrite (as NO_2^-) mg/L	0.08
19	Nitrate (as NO_3^-) mg/L	5
20	Chloride (as Cl^-) mg/L	337
21	Fluoride (as F^-) mg/L	Nil
22	Sulphate (as SO_4^{2-}) mg/L	213
23	Phosphate (as PO_4^{3-}) mg/L	0.04
24	Tidy's test (as O) mg/L	12.4
25	Silica (as SiO_2)	10.74
26	Total Kjeldahl Nitrogen as 'N' mg/L	82.88
27	COD mg/L	130
28	BOD mg/L	40
29	Chromium (as Cr) mg/L	0.0153
30	Copper (as Cu) mg/L	0.00714
31	Zinc (as Zn) mg/L	0.090

concentrations of medium such as (0, 25, 50, 75 and 100%). 0% was used as positive control, were water alone is utilised and 100% effluents as additional control, to understand the actual effects of effluent alone were treated as controls. The initial OD was recorded and also the final OD was calculated on the 5th day and 10th day. Fungi bioremediated effluent was centrifuged and supernatant was used as foliar sprays (sprayed twice) on *Solanum nigrum* plants. First spray was given on 15th day after seedling growth and a second treatment was given on the 30th day.

Pot A - Control only with water (0% effluent)

Pot B - Supernatant of (25 mL Effluent bioremediated with fungi + 75 mL of medium)

Pot C - Supernatant of (Fungi in 100 mL medium)

Pot D - Untreated effluent (Additional control)

After 30 days, the morphological and biochemical parameters such as height of the plant and length of the leaf and chlorophyll (Mackinney, 1941), carbohydrates (Dubois *et al.,* 1956) protein (Lowry *et al.,* 1951) and lipid (Barnes and Blackstock, 1973) were determined.

RESULTS AND DISCUSSION

The physico-chemical analysis of the textile effluent was analysed based on the parameters like pH, temperature, conductivity, turbidity, total solvents, Biochemical oxygen demand, Chemical Oxygen

Table 2 Growth of *Aspergillus niger* in different concentrations of the effluents was studied by fresh weight/dry weight method

S.No.	% of Effluent	Fresh weight	Dry weight	Fresh weight/ Dry weight
1	0	3.2 g	1.5 g	2.1
2	25 *	0.8 g	0.4 g	2.0
3	50	0.5 g	0.3 g	1.66
4	75	0.5 g	0.3 g	1.66
5	100	0.4 g	0.2 g	2

*** This concentration was taken up for further studies.**

demand, alkalinity etc. Water appeared turbid and also had an offensive smell. Temperature did not show much variation with that of atmospheric temperature. It was around 27ºC. The textile effluent showed a pH of 7.41. Turbidity was very high upto 85.3. Total solids were found to be high and above the permissible limit. This was mainly due to total dissolved solids which were found to be 1640 mg/L. Of the metals tested, sodium was high of about 370 mg/L followed by calcium (84 mg/L). Non-metal such as chloride was found to be high in the textile effluent than sulphate and total nitrogen. The increased level of sodium and chloride salts reflected in high concentration of total solids. Nutrients such as nitrite nitrate and phosphate were seen in minimum levels showing that the effluent water did not support the growth of fungi. So, the effluent was diluted with the medium at different concentration to study bioremediation. Chemical oxygen demand was found to be high when compared to Biochemical oxygen demand which reflected in Total dissolved solids. The high levels of Biochemical oxygen demand, Chemical oxygen demand and sodium chloride salts are due to the presence of dyes used in the textile industry. The heavy metals were analysed in atomic absorption spectrophotometer.

Of the metals tested, zinc was found to be higher than chromium and copper. The order of toxicity of heavy metals in the effluent water was Zn >Cr >Cu. (Table-1).

Growth of *A. niger* was measured using fresh weight / dry weight method. It showed that fungi grown in medum (0%) was high (2.1) and grown in 25% effluent was comparable to control (2.0). This concentration of fungal bioremediated effluent was taken for further studies and used as a foliar spray for S.nigrum plants (Table-2). The similar observation regarding the Fungal growth and biomass production in waste wood treated with chromate copper arsenate and ammoniacal copper quaternary has been observed by (Illman and Yang, 2010).

The result on Dissolved Oxygen (D.O) of mycoremediated effluent showed a negative correlation at the initial phase. Then the D.O increased on 5th and 10th day. The effluent bioremediated at 25% concentration was comparable to control. So it was used for further studies (Table-3). Similar results were observed in the studies performed by (Masud Hossain and Das, 2002 and Saritha *et al.,* 2010).

The textile waste water mycoremediated by fungi enhanced the morphological growth of *Solanum nigrum* Linn. on the first 15th day and second 15th day of foliar spray on all the 3 pots. However the mycoremediated effluent (Pot B - 25 mL Effluent bioremediated with fungi + 75 mL of medium) was comparable to control in parameters such as, height of the plant and length of the

Table 3 Dissolved oxygen of fungal bioremediated effluent before foliar spray

S.No.	% of Effluent	DO values			pH
		Initial	5th day	10th day	
1	0	-0.8	11.8	65.9	8
2	25	-2	11.6	68.3	8
3	50	1.3	10.5	20.5	8
4	75	-1	10	55.2	8
5	100	-2.5	8.7	10.2	8

Table 4 Effect of fungi and treated effluent on the height of the plant, after 15 days of the first and second foliar spray

S. No	Day observed	15 days of the first foliar spray				15 days of the second foliar spray			
		Pot A (cms)	Pot B (cms)	Pot C (cms)	Pot D (cms)	Pot A (cms)	Pot B (cms)	Pot C (cms)	Pot D (cms)
1	1st day	2.2	2	2	2	2.5	2.8	3	2.3
2	3rd day	2.2	2	2	2	2.9	3.1	3.2	2.3
3	6th day	2.3	2.1	2.2	2	3.3	3.4	3.5	2.3
4	9th day	2.5	2.3	2.3	2.2	3.8	3.9	4	2.3
5	12th day	2.6	2.4	2.4	2.3	4.2	4.3	4.5	2.3
6	15th day	2.8	2.6	2.5	2.4	4.8	4.9	5	2.3

Table 5 Effect of fungi and treated effluent on the length of the leaf, after 15 days of the first and second foliar spray

S. No	Day observed	15 days of the first foliar spray				15 days of the second foliar spray			
		Pot A (cms)	Pot B (cms)	Pot C (cms)	Pot D (cms)	Pot A (cms)	Pot B (cms)	Pot C (cms)	Pot D (cms)
1	1st day	1.2	0.9	1	0.7	1.6	1.6	1.4	1.1
2	3rd day	1.2	1	1	0.9	2	1.8	1.4	1.1
3	6th day	1.3	1.1	1.1	1	2.9	2.5	2.4	1.2
4	9th day	1.5	1.2	1.2	1.1	2.5	2.6	2.5	1.3
5	12th day	1.6	1.3	1.2	1.1	3.5	3.5	3.5	1.3
6	15th day	1.6	1.5	1.3	1.1	5	4	4	1.5

leaf after first and second foliar sprays (Table 4 and 5). Similar observation has been reported by (Saravanamoorthy and Ranjitha Kumari, 2005; Kannaiyan, 2001; and Sahai *et al.,* 1983) in *Arachis hypogaea* L. and *Phaselous radiatous* L. treated with distillery and textile industrial effluent.

Estimation of chlorophyll of the treated plant showed that mycoremediated effluent were comparable with that of control, while the raw effluent had a decrease in chlorophylls. Chlorophyll was found to be much affected due to raw effluent spray (Table-6).

The biochemical parameters such as analysis on carbohydrates, proteins, total lipids and chlorophyll a and b were investigated and the result showed that it is comparable to control. This suggested that mycoremediated water of textile effluent can be used equivalent to normal water for the favourable growth and nutrient levels of the plants. These findings are in agreement with earlier reports of (Swaminathan and Vaidheeswaran, 1991); Ramachandran, 1994; Veer and Lata, 1987; Jothimani *et al.,* 2002; Ramana *et al.,* 2002; and Saravanamoorthy and Ranjitha Kumari, 2005).

CONCLUSION

In this study it is observed that the mycoremediated water could be used as a suppliment to ground water. This indicates the degradation of textile waste water by using *A. niger*. is a natural and cost effective method. Considering this facts, the mycoremediated water could be reused for domestic and irrigation purpose. Further studies can be focused on enzymatic analysis of dye degradation using fungi. Water scarcity remains a major problem in Tamil Nadu, mycoremediated water can be utilized for irrigation of economically important ornamental crops.

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Table 6 Estimation of Chlorophyll, Carbohydrates, Proteins and Lipids after 15 days of second foliar spray.

S.No.	Pots	Amount of Chlorophyll a µg/mL	Amount of Chlorophyll b µg/mL	Carbohydrates µg/mL	Proteins µg/mL	Lipids µg/mL
1	A	199.335	396.05	42	12.4	2.70
2	B	198.456	382.32	38	12.2	2.72
3	C	197.676	386.35	36	12.2	2.19
4	D	152.980	316.10	24	10.4	2.15

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