POTENTIAL USES OF IMMOBILIZED CELLS OF *Fusarium oxysporium* IN THE BIOSORPTION OF HEAVY METALS FROM TEXTILE WASTEWATER

GARBA HARUNA
Department Of Civil Engineering, Nigerian Defence Academy Kaduna

Abstract
The effectiveness of immobilized cells of Fusarium oxysporium in the treatment of textile effluent as well as their ability to biosorp heavy metals was investigated. Textile wastewater from United Nigerian Textile Limited was seeded with F oxysporium cells in order to study their ability to reduced its pollution load before discharging into receiving water. Immobilized biomass of F oxsporium was inoculated into the textile effluent for 14 days and its efficiency in the treatment of textile wastewater was determined by the observed reductions in Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), as well as the removal of heavy metals after 14 days. During the retention time, a removal efficiency of between 42% to 95% for BOD, and 9.0% to 93.3% for COD was achieved. The biosorption capacities of F oxysporium for seven heavy metals (Co,Pb,Cd,Zn,Mn,Fe and Cr) was demonstrated. The percentage removal for heavy metals is as follows: cobalt 100%, manganese 90%, iron 36%, chromium 92%, cadmium 50% , lead 35% and zinc 81%. From these results it may be concluded that F oxysporium has some potentials in the treatment of textile wastewater especially in the removal of heavy metals.

Key words: biosorption, immobilized, degradation, wastewater, effluent, F*usarium oxysporium.*

INTRODUCTION

Textile industries are found in most countries and their number have increase considerably. These industries have shown significant increase in the use of synthetic complex organic dyes as colouring materials. The annual world production of textiles is about 30 million tones requiring 900,000 tones of different dyes, there by causing considerable environmental pollution problems (Zollunger 1987). Wastewater from textiles mills are acidic, colored, containing high BOD,COD and heavy metals are often discharged into nearby steams untreated, this causes the contamination or pollution of natural water and the environment, making it harmful to aquatic organisms and man (Fair et al 1971). The textile industries located within Makera and Kakuri drain are responsible for 64% of flow and 84% of COD and BOD loading of Kaduna river Beecoft (1994). Product of textile mills according to (Sidney 1978) are primarily fabrics with cotton as the main raw material. In many industries, water is commonly used

and little or no attention is paid to the cost of its recovery in the wastewater treatment plant where they exist. The control of water use which should be part of the general concern for Good Management practice is not employed. According to Fair et al (1971) textile wastewater could be treated biologically and that also colour could be removed by bleaching or adsorption, using suitable mechanisms.

MATERAILS AND METHOD

Textile Wastewater

The textile effluent used for this research was collected at a point of discharge from the textile mill in a plastic container on hourly basis for 10 hours (from 8am to 6pm) and mixed thoroughly before the analysis. They were stored at a temperature of $4^{o}C$ in a Refrigerator.

Equipments:

The following equipments were used:

i. UNICOM 929 Atomic Absorption Spectrometer was used for detection of heavy metals.

ii. A reflux apparatus manufactured by Gallenkamp England was used for COD determination.
iii. A carbon dioxide cooled temperature incubator manufactured by Gallenkamp England was used for BOD determination.

Organism:
The cells of the culture was obtained from the Department of Biological Sciences Ahmadu Bello University Zaria, which was Cultivated by modified Anderson method (Anderson et al 1973). The *F oxsyporium* cells was cultured on a medium consisting of the following (%w/v) : Malt extract 3.36, KH_2PO_4 0.001, NaH_2PO_4 0.002 $MgSO_4.7H_2O$ 0.005, dimethyl succinate 0.0015, thiamine hydrochloride 0.1, $FeSO_4.7H_2O$ 1.0, $ZnSO_4.7H_2O$ 1.0, $CuSO_4.5H_2O$ 2.0, H_3BO_4 2.0 and distilled water 10.0.

METHODS
Cell Immobilization:
The cells of the exponentially growing mycelia, sub cultured, was immobilized according to the following procedure, described by (Lee 1992).
i. About 0.55g of sodium alginate, 5ml of culture medium and 25ml of water were mixed and sterilized.
ii. The 30ml of suspension culture was allowed to settle and the concentrated cells was consigned at 20^0c.
iii. The alginate mixture was mixed with the concentrated cells.
iv. The mixture was pumped through a sterilized silicon tube (1.6ID) and feed drop wise into a flask containing sterilized 200ml of .12M $CaCl_2$, and was allowed to settle for one hour for complete precipitation, forming spherical beads of about 3.75-4.5mm diameter. The immobilized cells were removed and stored in distilled water at 4^0C.
Seeding:
About 10 liters of the textile effluent was poured into a model pond and about 1000 beads of the cells were introduced into the pond. The pond was mounted on a Jar test apparatus. The Jar test apparatus was able to stir the pond content so that the cells can move round the pond, a similar set up without seeding the sample served as control. Samples were taken every 24 hours for analysis. Parameters measured include BOD, COD and concentration of some heavy metals (Co,Pb,Cd,Zn,Mn,Fe and Cr).

BIOCHEMICAI OXYGEN DEMAND:
The diluted samples was taken in two bottles and the dissolved oxygen in one bottle was determined immediately, and the other after 5 days of incubation at 20^oC.The method used for BOD determination was the Standard Method for Examination of Water and Wastewater.
BOD was then calculated from $BOD=\frac{(D_1-D_2)\times B}{A}$ (mg/l)
Where
D_1= dissolved oxygen of sample in mg/l at start of experiment
D_2= dissolved oxygen of sample after 5 days
A=ml of sample before dilution
B= ml of sample after dilution

CHEMICAL OXYGEN DEMAND:
The COD is usually defined as the amount of oxygen used while oxidizing the organic matter of the sample with a strong chemical oxidant under acidic conditions. In measuring COD, the following steps were followed.
i. About 50ml of the sample was placed in a 500ml reflux flask and

75ml of $AgSO_4$ reagent was added.
ii. 25ml of $K_2Cr_2O_7$ solution was added and mixed thoroughly. The mixture was fixed on a condenser and reflux for 2 hours, it was cooled and diluted to 300ml.
iii. 2 to 3 drops of ferroin indicator was added and titrated against FAS until there was a sharp colour change from blue to green then to reddish brown the reading was recorded as volume of FAS used as (A ml).
Blank Titration
i. about 50 ml of distilled water measured into a reflux flask and 1g of were added.
ii. the flask was immersed in cooled water bath.
iii. 75 ml of $AgSO_4$ and H_2SO_4 reagents, about 25 ml of $K_2Cr_2O_7$ solution were added and mixed thoroughly.
iv. the mixture was reflux for 2 hours.
v. 2-3 drops of ferroin indicator was added and titrated against FAS until there was a colour change from blue green to reddish brown.
vi. the volume was recorded as FAS used for blank (B ml).

COD was calculated from

$$COD = \frac{(B-A)\times 8\times 1000(mg//)}{4\times 50}$$

ATOMIC ABSORPTION SPECTRO METER:
About 100ml of the wastewater sample was transferred into a beaker, 5ml of conc HNO_3 and glass beads were added to aid boiling. After boiling, the mixture was concentrated about 10ml to 20ml. the beaker was then washed with deionizer water and then filtered. The filtrate was transferred into a 100ml volumetric flask cooled and diluted to 100ml and mixed thoroughly, portions were then taken and the seven heavy metals were detected. For available metal determination, 0.5m Hcl was heated with the wastewater and reduced to about 10ml. The seven heavy metals are detected for each metal with its cathode lamp.

RESULTS AND DISCUSSIONS:
The characteristics of UNTL effluent wastewater was as shown in Table 1. From the result, the BOD, COD and heavy metals are above the acceptable limit and need remediation. The raw and seeded samples were analyzed. The results of the treated samples are shown in table 2.

Characteristic of Raw Textile Wastewater from UNTL Kaduna (concentrations in mg/l)

	Raw water	FEPA Standard
pH	9.6	6-9
Turbidity(Units)	70	-
BOD(mg/l)	380	30
COD(mg/l)	2990	150
Cobalt(mg/l)	1.176	0.5
Lead(mg/l)	1.076	-
Cadmium(mg/l)	0.018	-
Manganese(mg/l)	1.515	-
Iron(mg/l)	2.394	-
Chromium(mg/l)	4.744	0.01

Biochemical Oxygen Demand

The BOD removal efficiency for the inoculated and control samples are shown in figure 1 below

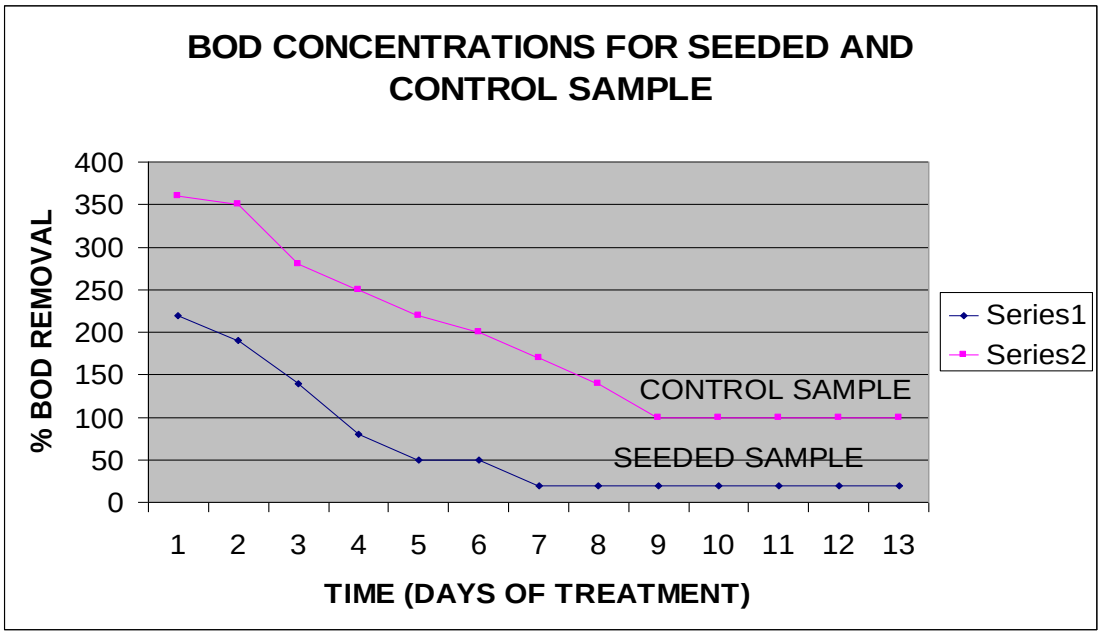

Fig 3.1 Time course of BOD reduction obtained by liquid cultures of *F oxysporium*

F oxysporium, strain was selected in this study on the basis of its previously reported work in: Fungal Diversity and Bioremediation (Sullia 2003). The result showed an observed reduction in BOD of 96% at 9 days of treatment for seeded sample. This is possible because the action of micro organism on organic wastewater lead to the gradual conversion of the wastewater into more micro organism until the waste water is exhausted.

Chemical Oxygen Demand

The COD removal efficiency for the inoculated and control samples are shown in figure 2

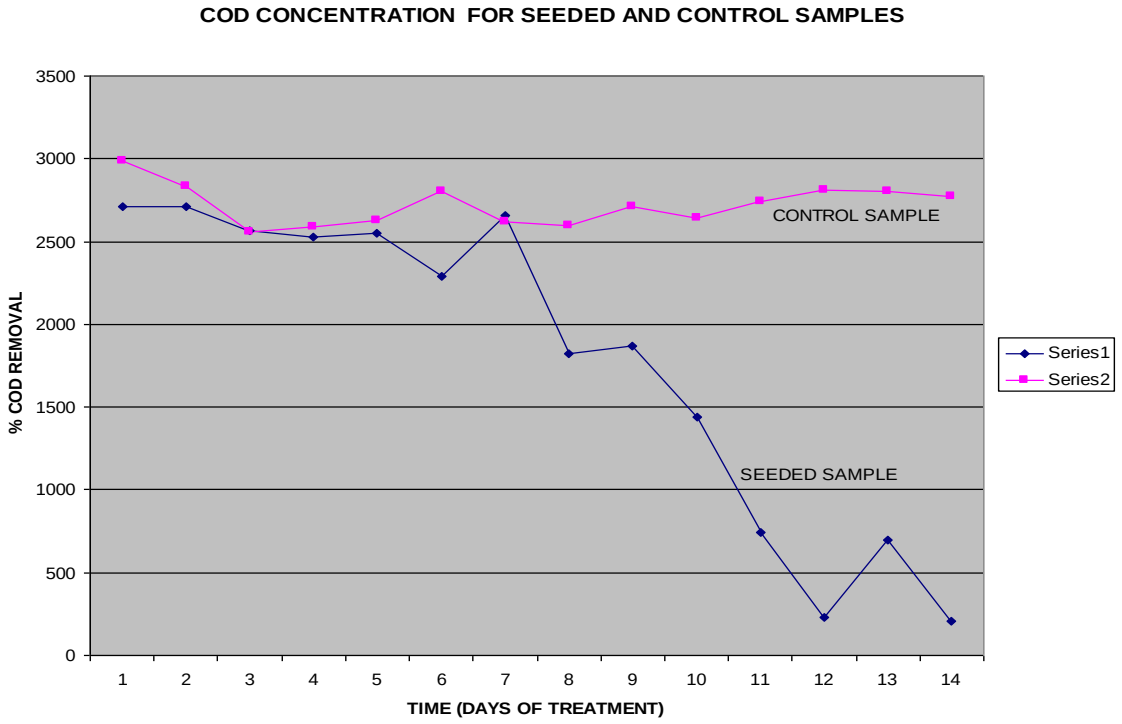

Fig 2 Time course of COD reduction obtained by liquid cultures of *F oxysporium*

The ability of *F oxysporium* in the treatment of textile waste is as depicted in fig 2. In the fig, there was an observed reduction in COD of 93% at 8 days of treatment for the seeded sample while the control experiment shows a percentage removal of 15% at 13 days. The ability of fungi to transform a wide variety of hazardous chemicals aroused interest in using them in bioremediation (Alexander 1976).

HEAVY METALS

TABLE 3.2 Heavy Metals concentration (mg/l)

Metal	**Initial concentration**	**Final concentration at 14 days**	**% removal efficiency**
Cobalt	1.176	0.00	100%
Lead	1.076	0.969	35%
Cadmium	0.018	0.009	50%
Zinc	1.094	0.207	81%
Manganese	1.515	0.512	90%
Iron	2.399	1.523	36%
Chromium	4.744	0.339	92%

Fungi are good in the accumulation of heavy metals such as cadmium, copper, mercury, lead and zinc,(Sullia 2003). The results of the experiment for determination of heavy metals at the beginning, and end of 14 days is as shown in table 3.2. *F oxysporium* ability to secrete extra cellular enzymes could be responsible for the accumulation of the metals. The maximum removal observed at 14 days treatment showed Cobalt and Chromium achieving 100% and 92% removal efficiency respectively. The Order of activity is Co

It is observed that the organism is not effective in the removal of lead but very active for cobalt.The order of activity is Co≥Cr≥Mn≥Zn≥Cd≥Fe≥Pb

CONCLUSION

The results obtained showed the ability of immobilized cells of *F oxysporium* to remove colourless organic substance and biosorp heavy metals from textile effluent. However the experiment could not identify the specific enzymes responsible. These results could be used to developed on site treatment system for textile processing industries effluent to achieved degradation of residual dyes as well as biosorption of heavy metals.

ACKNOWLEDGEMENTS

The author wish to thank Prof CA Okuofu, Amadu Bello University Zaria, for his suggestions and comments, Dr DB Maikaje Nigerian Defence Academy Kaduna for his valuable contribution, Dr PA Wuyep Ahmadu Bello Zaria for kindly assisting me with *F oxysporium* cells, Mr Ibrahim of College of Chemical and Leather Technology Zaria.

REFERENCES

Alexander M (1994). Biodegradation and Bioremediation. Acad. Press, San Diego, Calif, pg 4-10.

Ashoka C, Geetha MS and Sullia SB (2002). Biological Traetment of Combined Textile dye effluent in: Ecology of Fungai, DJ Bhat and Raghumar (eds), pg 63-67.

Anderson NA, Wang SS, Schwandt JW (1973). The Pleurotus Ostreatus Sapido Specie Complex. Mycologia 6:pg 28-35

Beecroft GA (1994). The Threat of Pollution to Rural Water Supply. A paper presented at Water Week Celebration, National Water Resources Institute Kaduna Pg 2.

Edmund BB and Max S(1976). The Treatment of Industrial Waste Second Edition Mc Graw-hill Inc London.pg 245-255.

Fair GM, Geyer JC, and Okun DA. (1971). Elements of Water Supply and Wastewater Disposal. Second Edison John Wiley and Sons inc New york

Garba H (2006). Uses of immobilizd cells of F oxysporium in Textile Wastewater Treatment. Unpublished Msc Thesis Department of Water Resoures and Environmental Engineering Ahmadu Bello University Zaria.

Sullia SB (2003). Fungai Diversity and Bioremediation in: Foundation For Biotechnology Awareness and Education Pg 3-4.

Sidney GC (1978). The Textile Industry Environmental Control And Energy Conservation, Noyes Data Corporation Park Ridge New Jersey USA.

APPENDIX 1

The Federal Environmental Protection Agency (FEPA) Standard for discharge of effluent into surface water

Parameter	Limit for discharge into surface water (mg/l)
Ph	6-9
BOD	30
COD	250
Total suspended solids	30
Oil and Crease	10
Pesticides	0.5
Chromium	0.5
Cobalt	0.5
Nickel	0.5
Zinc	0.5
Phenol	2
Sulphide	0.5

APPENDIX11

BOD concentrations (mg/l)

Treatment period (days)	Seeded sample	Raw sample
1	220	360
2	190	350
3	140	280
4	80	250
5	60	220
6	50	200
7	50	170
8	50	140
9	20	130
10	20	130
11	20	120
12	20	110
13	20	100
14	20	100

APPENDIX111

COD concentrations (mg/l)

Treatment period (days)	Seeded sample	Raw sample
1	2710	2990
2	2710	2830
3	2560	2560
4	2530	2590
5	2550	2630
6	2290	2800
7	2660	2620
8	1820	2600
9	1440	2710
10	742	2640
11	230	2740
12	200	2810
13	200	2800
14	210	2770