Scholars Academic Journal of Biosciences (SAJB)
Abbreviated Key Title: Sch. Acad. J. Biosci.
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A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

ISSN 2347-9515 (Print)
ISSN 2321-6883 (Online)

Plant Science

Phytoremediation of Grease in Wastewater Using *Dracaena sanderiana*

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Original Research Article

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Article History
Received: 02.04.2018
Accepted: 09.04.2018
Published:30.04.2018

DOI:
10.36347/sajb.2018.v06i04.007

Abstract: Industries like paper, pulp; food-processing and food restaurants and garages generate grease containing wastewaters for which there is no acceptable technology of pre-treatment. Most wastewater treatment technologies are known as being costly and not affordable by the municipalities and the small scale polluting industries. It is found that one of the effective methods of removal of toxic substances from soil and water using plants was developed which is known as phytoremediation. When land is available, phytoremediation is a promising and cost effective approach for domestic and industrial wastewater treatment. In the present work, aquatic plant *Dracaena sanderiana* (Lucky bamboo) used for the phytoremediation of grease in wastewater. Using microcosm approach, during remediation process grease wastewater was analyzed for parameters such as pH, color, turbidity, TSS, TDS, TS, fatty acid content and lipase activity. Significant change in color, pH, turbidity, TSS, TDS, TS and fatty acid content was observed. The current finding indicates promising use of *Dracaena sanderiana* for the phytoremediation of wastewater.
Keywords: Grease, lipase, phytoremediation, TS, wastewater.

INTRODUCTION

Domestic and industrial waste is the most common cause for water pollution. Domestic waste contains Solid food particles; oil and grease stick inside of the pipe which clogs the pipes. Organic matter, washing soap, detergents, high organic, suspended solids, oil and grease which cause harm to the environment and human health.

Pollutants can also affect the ground waters, when water is contaminated with organic matter it provides food for the mosquito larvae, which cause large increase in their population and they cause serious diseases in humans and animals. Drinking contaminated water can cause serious health problems like diarrhea, cholera, typhoid, dysentery and other illnesses such as guinea worm disease. It is important to control domestic wastewater for the betterment of the society and our future [1].

In wastewater generally fats, oil and grease (collectively called FOG) are also present. There are many misconceptions about methods and products for dealing with FOG. To make a wise decision, a better understanding of biological treatment versus chemical treatment is necessary. The main reason FOG is troublesome is that it is not water soluble. It eventually separates from water. FOG is lighter than water, so it floats to the top [2]. If not treated FOG molecules combine to form a hard layer and combines with other materials such as soap residue, paper and solids makes it worse. When this happens, a very hard crust can form on the surface of the water. The untreated FOG layer could flow out to the drain field and cause drain field problems such as grease traps, drain lines, and sewers can become clogged. Usually, an overload of FOG is what causes stoppages in flow [3].

There is a pre-treatment for removal of FOG is necessary because if the FOG will present in wastewater, it affects the other process of wastewater treatment. It also prevents oxygen supply for other processes. Different solvents and surfactants can be used for FOG degradation. They turn hard FOG into liquid state without changing its actual character. They do this by temporarily breaking the bonds of molecules (not the fatty acid and glycerides of the molecules) so they dissolve in water [4]. The water then carries the FOG downstream until the solvent wears off, at which point FOG reforms. High temperature also degrade FOG into soluble forms but when the temperature get down the FOG reforms so in both the process when the broken up fats in the water are passes, it causes blockages [5].

Plants can degrade, extract or stabilize a contaminant in wastewater, thus making it unavailable for other organisms and reducing environmental hazards [6]. If the contaminant in its present concentration is not phytotoxic, cultivation of plants can be a valuable tool in wastewater remediation. Mechanism and efficiency of this technology called phytoremediation, depending on the type of contaminant, bioavailability and wastewater properties [6]. It is an economic method to reduce contaminant load in wastewater. It involves the use of green aquatic plants *Eicchornia crassipes* (water hyacinth), *Nymphaea* sp (water lilly), *Lemnoideae* (duckweed), *Canna indica* (water canna), *Dracaena sanderiana* (lucky bamboo) and many more [7].

The current work deals with phytoremediation of grease in wastewater using aquatic plant *Dracaena sanderiana* (lucky bamboo). The finding indicates effective use of bamboo plant for remediation of pollutant present in wastewater.

MATERIALS AND METHODS

Media and Chemicals

Tributyrin agar (composition in g/l)*:* Peptic digest of animal tissue 5 g, Yeast extract 3 g, Agar 15 g, Tributyrin 100 ml, water 1000 ml, pH 7.5. *1% Phenolphthalein*: 0.1 g Phenolphthalein indicator was added into 10 ml 95% ethanol. *0.1M Potassium hydroxide (KOH):* 0.56 g KOH was prepared in 100 ml 95% ethanol.

Collection of plant

The plant Lucky bamboo (*Dracaena sanderiana*) was bought from nursery near Kalupur railway station, Ahmedabad.

Collection of wastewater sample

The sample of greasy wastewater was collected from the Ahmedabad- Mehemdabad highway.

Plant set up for phytoremediation

Plant was set up for phytoremediation was prepared into glass beakers as following combinations:

A: Plant was kept in a glass beaker containing 150 ml fresh water, known as control.
B: Plant was kept in a glass beaker containing 150 ml greasy wastewater.
C: Plant was kept in a glass beaker containing 150 ml greasy wastewater containing 400 ppm of Indole Acetic Acid (IAA).
D: Plant was kept in a glass beaker containing 150 ml greasy wastewater containing 400 ppm of Gibberellic acid.
E: 150 ml greasy wastewater was kept in a beaker without plant as abiotic control.

The whole set up was kept at 25^{o}C under laboratory conditions for 10 days. At the interval of 48 h grease water sample was analyzed for different parameters.

Estimation of Fatty acid content

The degradation of grease was calculated in terms of free fatty acid released in wastewater. 2 ml of wastewater sample was taken in flasks from each samples and then 10 ml of solvent mixture (1/1, v/v 95% ethanol / diethyl ether) was added. 5 drops of 1% phenolphthalein indicator was added to the flasks. The mixture was titrated with 0.1M KOH solution [8]. The initial and final burette readings were note down and the fatty acid value was calculated by the following formula:

Fatty acid value (mg/ml) = 56.1 * N * V / M
Where, 56.1 = molecular weight of KOH
N = Normality of KOH
V = ml of KOH solution used
M = ml of sample

Estimation of Total dissolved solids (TDS)

2 ml of wastewater sample was taken and filtered through filter paper. The filtrate was collected in glass beaker and weighed as W_1. The water sample was allowed to evaporate and after that the beaker was weighed as W_2. TDS was calculated by the following formula:

TDS (mg/l) = $(W_2 – W_1)$ * 1000 / volume of sample (ml)

Estimation of Total suspended solids (TSS)

The filter paper was weighed as W_1 before filtration. After filtration the residues left on filter paper were evaporated and weighed as W_2. TSS was calculated by the same formula as for TDS:

TSS (mg/l) = $(W_2 – W_1)$ * 1000 / volume of sample (ml)

Estimation of Total solids (TS)

TS are the sum of TSS and TDS, which was calculated by the following formula:
TS (mg/l) = TDS + TSS

Estimation of pH, Color and Turbidity

The pH value was measured by pH strips, whereas the color of wastewater from each setup was done through visual observation. For turbidity wastewater at regular time interval absorbance was measured at 540 nm [14].

Lipase assay

Tributyrin agar were allowed to solidify and punched by sterile gel puncture. Punctures were filled by 100 µl of wastewater without plant, wastewater with plant's leaf extract, stem extract, root extract and distilled water (as control) and incubated at 37^{o}C for 24 hours. The plates with transparent zone around well were considered lipase positive [9].

RESULTS

Degradation of grease in wastewater using Dracaena sanderiana

The degradation of grease was estimated in terms of free fatty acid released during remediation. The wastewater with only plant showed the highest degradation of grease over the duration of 10 days. In the presence of IAA and GA, fatty acid content gradually increased till 6 days but afterwards decline in fatty acid content was observed. The wastewater without plant (negative control) showed the minimal degradation (Fig. 1). The amount of free fatty acid is directly proportional to the degradation of grease in wastewater. The maximum degradation of grease was observed in wastewater with plant; while the minimum degradation was observed in abiotic control, which may be due to some microbial activity. Further studies had also shown activity of microbes in degradation of oil and grease in wastewater [10].

The presence of hormones had no significant effect on grease degradation and the fatty acid value was first increased but later it decreased. This might be happened due to some rhizofiltration mechanism in which plant and microbes absorbed the degraded fatty acid molecules. Some previous studies showed that in plants, plants absorb fatty acid which plays significant roles in pathogen defense. They also play role as biosynthetic precursors for cuticular components or the phytohormones [11]. *Canna indica* and *Cyperus alternifolius* had shown upto 4 mg/l of oil and grease degradation within 24 hours [8]; while *Dracaena sanderiana* had shown about 3 mg/ml of grease degradation over the period of 10 days.

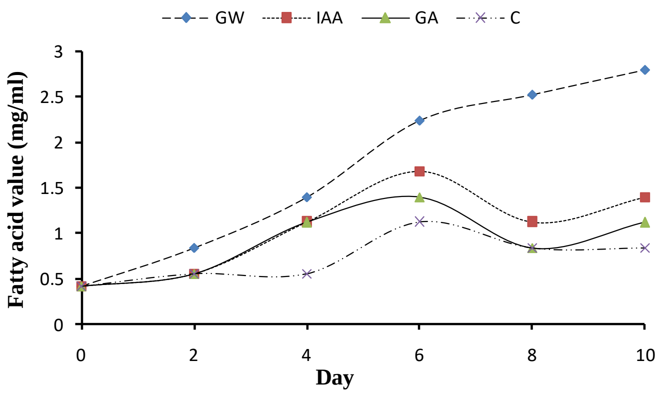


Fig-1: Change in Fatty acid content (mg/ml) of grease water under different conditions

Change in TDS, TSS and TS during phytoremediation of grease wastewater

The maximum decline in TDS was observed within initial 48 hours. After that there was no significant change in TDS under all studied conditions (Fig. 2). The decrease in TSS was not significant in initial two days. However, maximum decline in TSS was observed on day 4. The TSS content remained unaffected by experimental conditions and show similar content under all studied phytoremediation condition (Fig. 2). The value of TS significantly decreased within 4 days under studied phytoremediation conditions. Thereafter, no change in its amount was observed (Fig. 2).

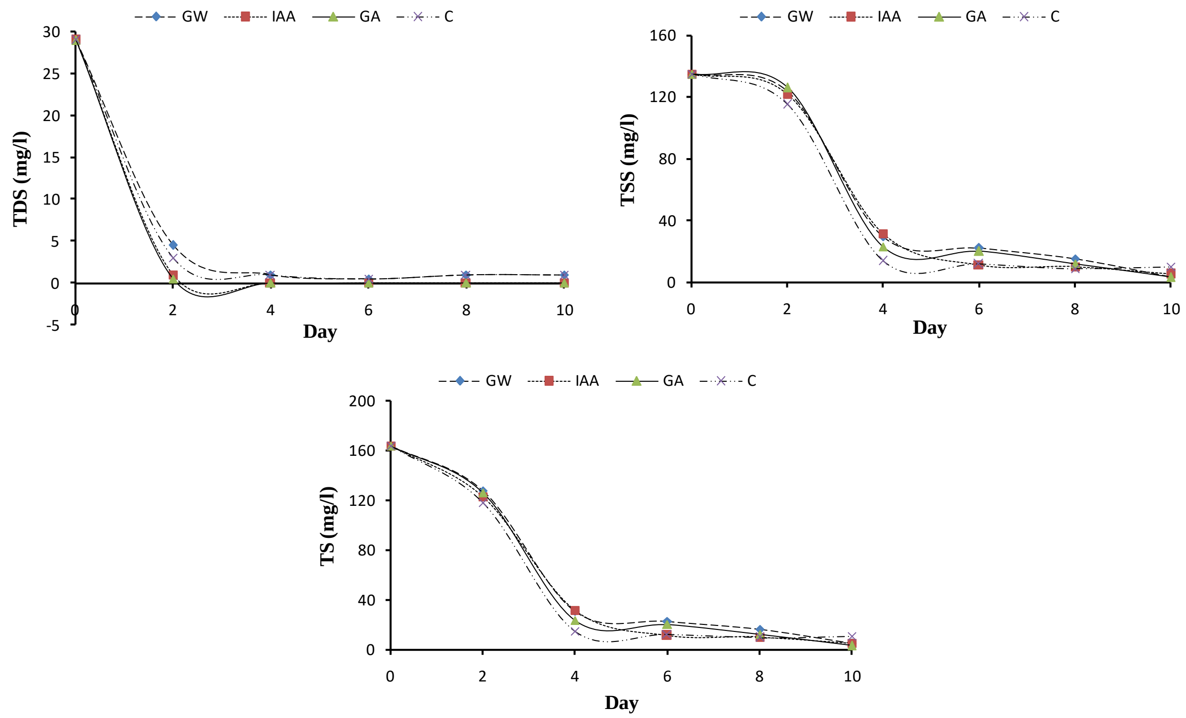


Fig-2: Change in TDS, TSS and TS (mg/l) of grease water over the period of 10 days under different phytoremediation setup

Effect of phytoremediation on pH of grease wastewater

Before degradation the pH of the grease water was 9. During the process of phytoremediation, in presence of *Dracaena sanderiana* the pH of grease water gradually decreased to 7. Similar finding was observed in presence of IAA and GA (Fig. 3). The initial pH of wastewater sample was 9 which indicated alkalinity of the wastewater. It might be due to presence of some detergents, solvents and chemical reagents which was also found in previous studies [12]. During the process of phytoremediation the initial pH fell down upto 7 and wastewater became neutral in all the sets with plant and hormones which showed significant decrease but in abiotic control there was no significant decrease and wastewater remained alkaline. The decrease in pH from 8.5 to 6.5 was observed using *Ocimum sanctum* in further studies [13]; while *Canna indica* and *Cyperus alternifolius* showed no significant decrease in pH [8].

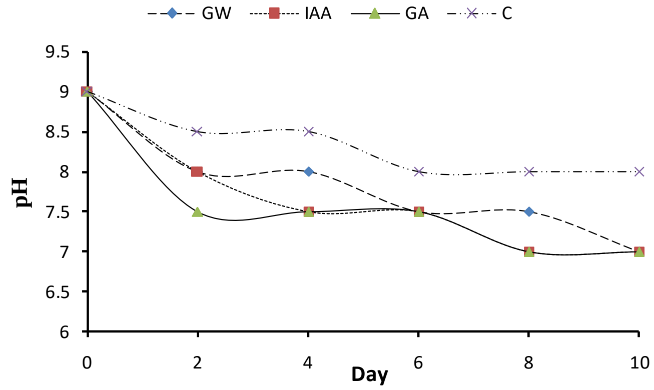


Fig-3: Change in pH during phytoremediation of grease water

Effect of *Dracaena sanderiana* on color and turbidity of grease wastewater

Initially the color of grease wastewater was grey, which gradually changed to translucent over the period of 10 days in presence of *Dracaena sanderiana* with IAA and GA, whereas the color change to trout in wastewater with *Dracaena sanderiana* and without plant.

The significant decrease in turbidity was observed in presence of *Dracaena sanderiana* with IAA and GA. The maximum change in turbidity as observed within 4 days (Fig. 4).

Color is the qualitative characteristic for the general condition of wastewater. Wastewater which was grey in color when collected from the site showed that the wastewater was typically septic and the grey color of wastewater was may be due to the formation of various sulphides; grease and some dissolved and suspended solids [13]. Presence of hormones indicated color change from grey to translucent which showed more aesthetic look of the water; while wastewater with plant and the abiotic control showed change in color from grey to trout. The change in color from grey to transparent was observed by *Canna indica* and *Cyperus alternifolius* for the treatment of wastewater [8]. The 70% of dye decolorization in wastewater was observed by *Lemnaceae* plants [14].

The turbidity of wastewater due to colloidal materials provides absorption sites for chemicals, dyes, reagents, etc; which increases the load on filters. This study showed 100% decrease in turbidity of grease wastewater in the presence of growth hormones; while 90% of decrease was observed in wastewater with plant and abiotic control showed minimum decrease. In previous study, about 50% of decrease in turbidity was observed using *Ocimum sanctum* [13].

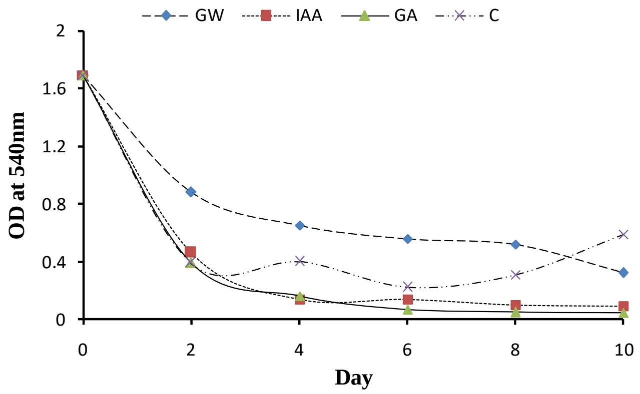
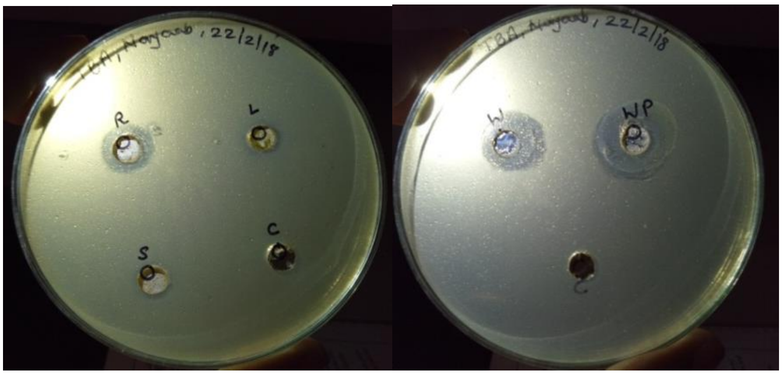


Fig-4: Change in turbidity of grease water during phytoremediation

Lipase assay

The grease wastewater with *Dracaena sanderiana* showed positive lipase activity. A clear transparent zone around well was observed. Root, stem and leaves part of *Dracaena sanderiana* were also tested for lipase. Lipase activity was observed in root extract of the plant. The finding indicates secretion of extracellular enzymes from plant, which further involved in grease degradation process (Fig. 5).

Lipases are the enzymes which degrade lipid into fatty acid and glycerol. The positive lipase activity on tributyrin agar was observed in root extract and in wastewater with plant which indicated grease degradation in wastewater by these enzymes. However stem and leaf of the plant also showed lipolytic activity but it found maximum in roots. The increased free fatty acid content showed permanent degradation of grease in wastewater. The lipase activity was also found in abiotic control but it was less than the biotic control. Similar findings were shown by the lipolytic bacteria isolated from soil contaminated with oil [9].



(A) **(B)**

Fig-5: Lipase activity on Tributyrin agar plates in (A) Plant parts extract (B) grease wastewater (R; root, L; leaf, S; stem, W; wastewater without plant, WP; wastewater with plant, C; negative control)

Change in *Dracaena sanderiana* appearance during phytoremediation of grease wastewater

During the process of phytoremediation, the plant appearance was observed on 0 and 10th day. There was no change in stem diameter. The height of the plant was changed from 11 cm to 11.7 cm. The change was observed about 4-7 mm (Table 1). There was significant change in number of buds before and after process. Initially, the number of buds were from 2-4, while after process it changed from 3-8. The maximum number of buds was observed in the presence of IAA (8 on 10th day). In the presence of GA the number of buds increased from 3 to 5.

Table-1: Effect on the physical appearance of the plant

Sets	Before process			After process		
	Height	Width	No. of buds	Height	Width	No. of buds
A	11	0.4	2	11.7	0.4	3
B	11	0.4	3	11.3	0.4	4
C	11	0.4	4	11.5	0.4	8
D	11	0.4	3	11.4	0.4	5

Dracaena sanderiana grew normally in fresh water; while plant placed in wastewater showed slower growth than fresh water but there was no negative effect of grease wastewater observed on plant physical appearance. However presence of hormones IAA and GA showed more growth than plant in wastewater without hormones. The change was observed in the length of the plant and in number of buds formation; while there was no change in plant width in all the setups. Some previous studies had shown increased salinity and reduced plant (*Zea mays*) growth using oil mill wastewater (OMW) effluent, although treated OMW enhanced plant growth compared with the untreated. The plant growth remained lower than that obtained using the potable water with fertilizers, indicated lack of some essential plant nutrients [15]. Another study showed some negative effects of kitchen wastewater on plant (*Cucumis sativus*) growth; leaf area and leaf number [16]. Use of aquatic plant *Dracaena sanderiana* in phytoremediation showed significant changes in values of fatty acid, TDS, TSS, TS, pH, color and turbidity in grease wastewater. Lipase activity for the degradation of grease into fatty acid and glycerol was observed in plant root and in wastewater.

The overall findings showed the effective use of *Dracaena sanderiana* for phytoremediation of grease wastewater without any negative effects on remediation process and plant's growth.

CONCLUSION

Phytoremediation method using *Dracaena sanderiana* for degradation of greasy wastewater is feasible. The above study indicates the use of plants for removing of contaminants from wastewater is cost effective and having a very less operation and maintenance work. Phytoremediation is economical as compare to other treatment methods. It is a natural process not harmful to the environment. It is effective on low strength contaminants and very easy method to operate. It is more effective method for removal of hazardous pollutants and for the removal dissolved nutrients. The phytoremediation mechanism has high removal rate of pollutants in wastewater. The treatment had also improved the qualitative characteristics of the wastewater such as color and turbidity. The treated water can be use for gardening and other related purposes because no negative effect was observed on plant growth and physical appearance. Phytoremediation has not been fully commercialized. Future studies should focus to develop more stable and efficient phytoremediation systems and microbe-plant combination systems. The use of genetic engineering technology will be a convincing approach to develop better strains of plant with higher remediation capacity.

ACKNOWLEDGEMENTS

Authors of the manuscript are thankful to the authorities of Government Science College, KK Shashtri Education campus for providing laboratory facilities to carry out research work.

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