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Biodegradation of tannery effluent dyes by using *Aspergillus niger* isolated from the tannery effluent and reuse of biotreated water in agricultural field

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Abstract

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater and land used for agriculture. The present research work has been carried out to isolate and screen the fungi from tannery effluent, evaluation of their ability to decolourize the dyes and reuse of biotreated water in agricultural purposes. The optimal dosage of coagulant used for treating tannery effluent was found to be 8%. Four fungal species were isolated and identified by LPCB staining namely *Aspergillus Niger, Aspergillus flavus, Penicillium citrinum* and *Curvularia lunata*. Among these *Aspergillus Niger* was found to be more effective in decolourization of dyes present in tannery effluent. Finally, the bio-treated tannery effluent was reused for agricultural purposes i.e., for the growth of vegetable plant *Cyamopsis tetragonoloba* and various growth parameters were studied.

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Introduction

The tannery effluent waste is ranked as high pollutants among all other industrial waste (Eye and Lawrence, 1971). Microbes in the environment play an important role in cycling and destroying them through biodegradation. In recent years a number of studies have focused on some microorganisms capable of degrading and absorbing dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolourization of dyes. It has been reported that microorganisms are capable of degrading dyes and could be used in effluent treatment plants for removal of these dyes (Ponraj *et al*., 2011; De-Bashan *et al*., 2017). Nearly 30m^3 of wastewater is generated during processing of one tone of raw skin (Suthanthararajan *et al*., 2014). Heavy metals are powerful inhibitors of biodegradation activities. These metals cannot be degraded. The toxic effects of heavy metals result mainly from the interaction of metals with proteins and inhibition of metabolic processes. These heavy metals such as copper, cadmium, zinc, lead, mercury, nickel and chromium when accumulated in soils are toxic to plants, animals, humans and aquatic life. For treatment of tannery effluent, various remediation options like use of chemical coagulants, oxidation ponds, trickling filters, activated sludge processes are present (Kaul *et al*., 2014). Chromium exists in two forms, trivalent and hexavalent, where hexavalent is more toxic than trivalent. Biological methods such as microbial degradation, adsorption by microbial biomass and bioaccumulation by growing cells are commonly applied to the treatment of industrial wastewater since many microorganisms such as bacteria, yeast, algae and fungi are able to absorb, accumulate and degrade different organic pollutants (Singh *et al*., 2015). Fungi, bacteria, yeast and algae are the types of microbes used for decolonization of dyes among which the major research appears to have been conducted on fungi and bacteria as they have the ability to decolorize dyes almost completely. Compared to bacteria and filamentous fungi, yeast has many advantages, they not only grow rapidly like bacteria but like filamentous fungi they also have ability to resist unfavorable environments (Yu and Wen, 2005). The main advantage of biological treatment in comparison with certain physicochemical treatment is that over 70% of organic matter expressed by CoCr may be converted to bio solids (Anjaneyulu *et al*., 2005). Disposal of such tannery waste with high pollution load into water courses with or without prior treatment creates a great problem in the environment in the vicinity. So, it is essential to treat the wastewater before disposal. In the present study, an attempt was made for the isolation and identification of fungi from the tannery effluent by LPCB staining, potential fungi strains for decolourization of tannery effluent containing dyes and reuse of biotreated effluent in agriculture (ie) growth of vegetable plant *Cyamopsis tetragonoloba a*nd estimation of amino acid, carbohydrate, fatty acid, protein and chlorophyll in the leaf of *Cyamopsis tetragonoloba*.

Materials and methods

Sample Collection

Tannery effluent was collected from Common Effluent Treatment Plant (CETP) Pallavaram, Chennai during March 2019. The effluent was brought to the laboratory and stored at 20^{o}C.

Effect of coagulant dosage

Five conical flasks, each with 500ml of tannery effluent was taken. Specified dosage of coagulant ranging from 6-10% of PAC was added and stirred rapidly for 10 minutes. Finally, the flocs were allowed to settle for 2 hours before withdrawing the supernatant for analysis. The coagulation formed was measured in cm and the maximum dosage of coagulated supernatant was taken for further studies. UV scanning was done with the supernatant and the highest wavelength (nm) absorbance was selected for further studies.

Isolation of dye degrading fungal isolates from tannery effluents

Fungal isolation was carried out by serially diluting the sludge formed after coagulation and plated on SDA medium by pour plate technique and incubated at 30°C for 3-5 days (Onions *et al*., 1995). After incubation, colonies were isolated and identified by LPCB staining.

Decolonization assay

The identified fungi were used for decolonization activity. Decolourization activity was expressed in terms of percentage and was determined by monitoring the decrease in absorbance at absorption maxima (max). 100ml of SD broth with 15ml of tannery effluent was sterilized at 121°C for 15 minutes. After sterilization, the fungal isolates were inoculated in the broth. The initial absorbance was taken and kept for incubation in shaking conditions at 30°C for 3-5 days. After incubation, the culture suspension was centrifuged at 8000 rpm for 10 minutes for removal of biomass. The degree of decolonization was measured at its respective maximum absorbance 480nm by using spectrophotometer. The decolonization assay was calculated according to the following formula (Chen *et al*., 2011; Ali *et al*., 2009). The isolate which showed more than 70% decolonization was selected for treating tannery effluent.

Reuse of bio treated effluent in agricultural field

After decolonization of dyes, the bio treated water was reutilized for the growth of vegetable plant *Cyamopsis tetragonoloba* and various growth parameters were studied for a period of 15 days and 30 days (Jerin, 2009). Comparative study of growth was done by using freshwater, bio treated water and untreated water.

Estimation of Chlorophyll

0.50 mg of fresh leaf material was homogenized with 10ml of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 minutes and the residue was extracted with 10ml of 80% acetone. The concentration of chlorophyll a, b and total chlorophyll were quantified in samples by reading the OD value at 630nm and 670nm (Arnon, 1947).

Estimation of amino acid

0.05g of plant leaf was weighted and homogenized with 10ml of 80% ethanol. The homogenate was centrifuged for 10 minutes and the ethanol is evaporated in water bath at 50°C and 1 ml of the extract was added to 1 ml of ninhydrin reagent. The content was heated in boiling water bath for 15 minutes, cool the test tubes in cold water and add 5ml of diluent solvent and incubate at room temperature for 10 minutes. The absorbance was read at 570nm using spectrophotometer (Moore and Stein, 1948).

Estimation of carbohydrate

To 1ml of plant leaf extract 5ml of anthrone reagent was added and the solution was kept in water bath for 10 minutes and cooled at room temperature. The OD value was taken at 620nm (Dubois *et al*., 1956).

Determination of fatty acid (Titration method)

0.05g of crude plant leaf extract was taken. To this 5ml of ethanol, 5ml of petroleum ether and 1 ml of phenolphthalein was added. KOH was used for titration against this crude extract. Pale pink color was formed and the reading was noted in the burette (Cox and Pearson, 1962).

Determination of protein (Lowry's method)

0.05g plant leaf was homogenized with 10ml of dis water. 1 ml of homogenized mixture was taken and mixed with 5 ml of alkaline copper reagent. Keep the solution in dark for 10 minutes and add 0.5ml of folin ciocalteau phenol reagent. The solution turns dark purple Colour and the OD value was taken at 750nm (Lowry *et al*., 1951).

Results and discussion

A significant proportion of dyes enter to the environment through wastewater. Microbial decolonization has been proposed as a less expensive and less environmentally intrusive alternative.

Effect of coagulant dosage

PAC is used for the treatment of drinking water, waste water treatment, treatment of sewage and industrial effluent. Coagulation is the process of adding and mixing a coagulant with colloidal substances and terminated by the formation of large sized particles. Coagulation used ahead of gravity settling may be expected to yield suspended solids removals of about 90%. The optimal dosage of coagulant used for coagulation was found to be 8%.

Isolation of fungal isolates from tannery effluent

The effluent is rich in organic and inorganic nutrients which would have supported the growth of fungal population. The fungal strains were isolated from untreated tannery effluent by serial dilution technique. The results indicated that some native fungi are adapted to heavy metals under constant metal stress for a long time and the toxic metals are used as micronutrients by the growth of fungi (Zhang *et al.*, 1951). The results of the microbial analysis of the effluent showed four species of fungi such as *Aspergillus Niger, Aspergillus flavus, Penicillium Citrinum and Curvularia lunata*.

Decolourization assay

The fungal isolates were screened for decolonization activity. After decolonization, *Aspergillus Niger* was screened out which showed more than 70% decolonization. After biodegradation, the effluent color changed from blackish to almost colorless nature, due to the action of microbes. *Aspergillus niger* which decomposed the toxic pollutants present in the effluent and changed the color and odor of the effluent (Krishna Priya, 1910). The decolourization was expressed as percentage (%) and estimated by the formula. The percentage of decolourization was shown in Fig 1. and Table 1.

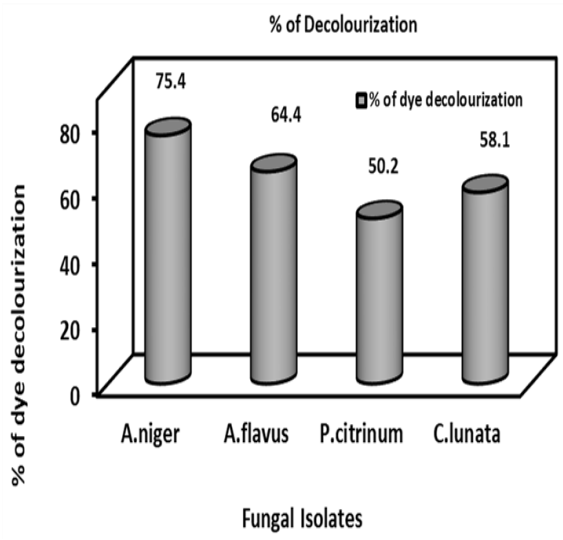

Fig. 1. The percentage of decolourization.

Table 1. % of dye decolourization by Fungal Isolates.

Fungal Isolates	*A.niger*	*A.flavus*	*P.citrinum*	*C.lunata*
% of dye decolourization	75.4	64.4	50.2	58.1

Estimation of Chlorophyll

Green color is important source of protective food which is highly beneficial for the maintenance of good health and prevention of diseases. Plant leaves were used to estimate the chlorophyll content. Chlorophyll estimation was done in the fresh green leaf samples extracted with the acetone solvent and the absorbance reading of chlorophyll extracts was measured in two different wavelength 630nm and 670nm respectively. Based on the absorbency value, calculations were made and the amount of chlorophyll a, chlorophyll b and total chlorophyll as shown Fig 2. The highest total chlorophyll content (a+b) after 15 days was detected in plant treated with freshwater 0.611nm followed by treated water 0.591nm. After 30 days, plant treated with freshwater contains 1.079nm followed by treated water contain 0.960nm. Estimation of chlorophyll is also studied by using medicinal plant (Rajalakshmi and Banu, 2014). Further isolation and spectroscopic analysis of natural pigments suggested the strong antioxidant capacities were attributed to chlorophyll derivatives. The results indicate that the decolorization process was able to improve the agar quality, and the extract containing lots of natural pigments had antioxidant activities which may be used in functional food and cosmetics.

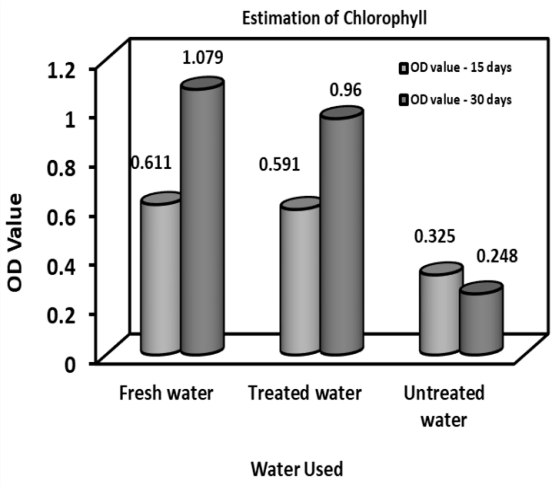

Fig. 2. Estimation of chlorophyll.

Estimation of amino acid

The total amino acid content after 15 days with plant treated with freshwater contain 0.205nm followed by treated water 0.198nm. After 30 days, plant treated with freshwater contain 0.261nm followed by treated water 0.231nm. The estimation of amino acid was shown in Fig 3. The amino acids and are essentially the basic component of all living cells Growing medium amino acids and mineral nutrients stimulate rhizosphere activities and plant growth. These require specific and adequate quantities of plant tissue amino acids with each or combination of the amino acids performing specific function. Root uptake of amino acids can vary with the concentration, plant genotype, and rhizosphere microbial activities while microorganisms and plant roots compete for the available amino acids This leads to an increase in chlorophyll concentration and the greening of onion leaf tissues because of the availability of free amino acids in the growing medium. The content of these secondary metabolites and nutrients may be influenced by the composition of growing medium free amino acids.

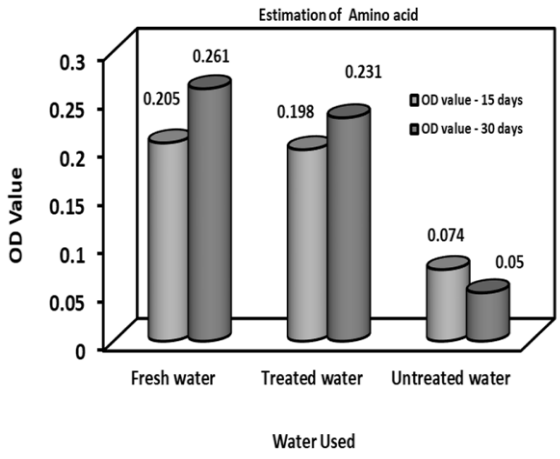

Fig. 3. Estimation of aminoacids.

Estimation of carbohydrate

On the basis of carbohydrate studies in treated leaves and exudates from excised leaves, it was suggested that carbohydrate accumulation in leaves might be caused by an impairment of phloem loading. This would explain the inhibition of photo assimilate translocation and may be part of the plant death mechanism, because meristematic tissues would be carbon starved. Alternatively, carbohydrate accumulation in leaves might be caused by a decrease in sink strength. Despite carbohydrate accumulation in leaves, there was no carbohydrate shortage in roots. Indeed, carbohydrates were also accumulated in roots. This decrease in sink strength seems to cause the carbohydrate accumulation in leaves. The total carbohydrate content of plant leaf was estimated on 15 days and 30 days. The carbohydrate content of plant treated with freshwater after 15 days was found to be 0.205nm followed by treated water 0.198nm. The carbohydrate content after 30 days in plant treated with freshwater was found to be 0.261nm followed by treated water 0.224nm. Estimation of carbohydrates was also studied from different parts of *costus speciosus* (Karabi *et al*., 2014). The estimation of carbohydrates was shown in Fig 4.

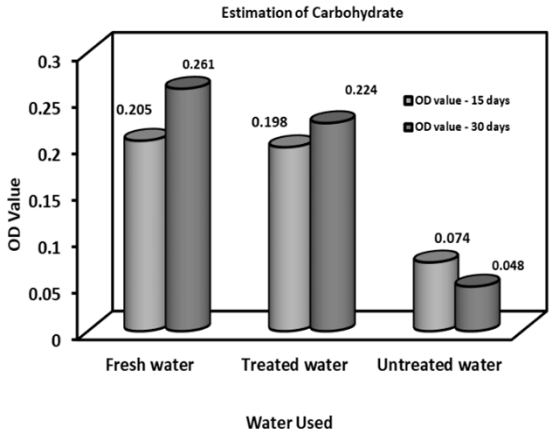

Fig. 4. Estimation of carbohydrate.

Estimation of fatty acid

This paper describes a method for manipulating plant membrane fatty acid compositions without altering growth temperature or other conditions. Treated plants incorporated large amounts of exogenous fatty acids into all acrylate membrane lipids detected. Fatty acids were taken up by both roots and leaves. Fatty acids applied to roots were found in leaves, while fatty acids applied to leaves appeared in both leaves higher on the plant and in roots was evaluated. Longer saturated fatty acids accumulated in plants treated with shorter chain of fatty acids. The fatty acid content of plant treated with freshwater after 15 days was found to be 0.5mg/ml followed by treated water 0.4mg/ml.

The plant treated with freshwater after 30 days was found to be 0.5mg/ml and treated water 0.5mg/ml. Estimation of fatty acid was shown in Fig 5.

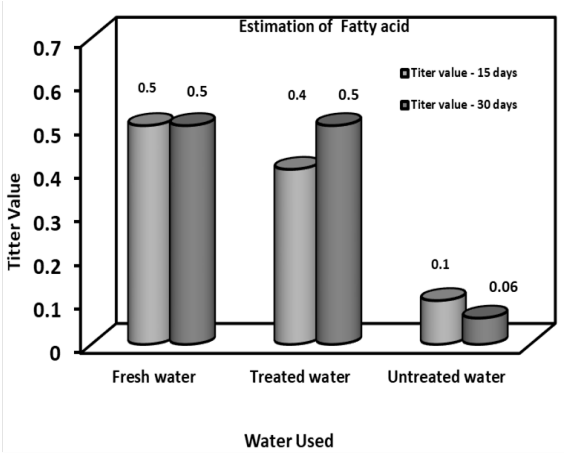

Fig. 5. Estimation of fatty acid.

Estimation of protein

The aromatic amino acid of Jatropha protein reacts with Folin - cioaclteau regent (Lowry's reagent) and form a complex which is blue purple Colour complex (Singh *et al.*, 2006). In Lowry's method the *Cyamopsis* extract showed the absorbance of the plant treated with freshwater after 15 days was 0.177nm and treated water as 0.150nm. After 30 days the absorbance of the plant treated with freshwater is 0.221nm and treated water is 0.204nm. The untreated tannery effluent showed decrease in the seed germination percentage of *Lycopericum esculentum* (Mandakini Magre and Khillare, 2016). Estimation of protein was shown in Fig 6.

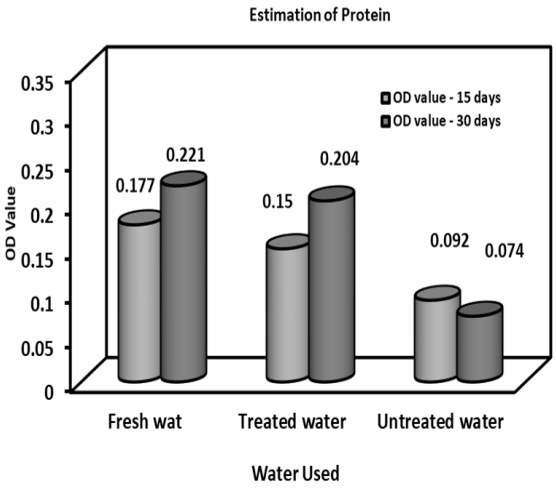

Fig. 6. Estimation of Fatty Acid.

Conclusion

The results obtained shows that *Aspergillus niger* isolated from the tannery effluent is the efficient strain to decolourize the dye present in the effluent. The presence of toxic substances present in the waste water has decreased the growth of *Cyamoposis tetragonoloba*. Whereas increased rate of germination and growth of *Cyamopsis tetragonoloba* in 100% biotreated sample is due to the maximum removal of toxic substances by native fungi, *Aspergillus Niger*. Thus from the above study, it is concluded that *Aspergillus Niger* has the degrading efficiency of the tannery effluent and in the present study it is evidenced that biotreated tannery effluent can be utilized for agricultural purpose.

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