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Utilizing *Borassus flabellifer* sprout peel sugars by *Pseudomonas fluorescence* for degradation of textile effluent

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Abstract

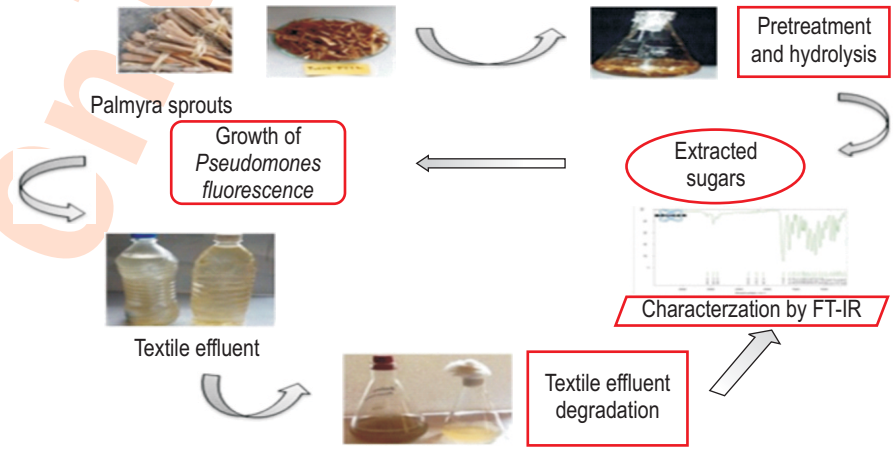
Aim : The present investigation deals with the extraction of sugars from tuber peels of *Borassus flabellifer* and their subsequent utilization for the growth of a bacterial isolate. The study also aims to degrade the textile effluent using *B. flabellifer* sprout peel sugar supplemented bacterial isolate.

Methodology : The isolate was screened from a textile effluent and was identified as *Pseudomonas fluorescence*. The sugar peels were pretreated by dilute acid hydrolysis and extracted sugars were used as supplement for the growth of *Pseudomonas fluorescence*. The textile effluent was treated with the bacterial isolate for degradation. The decolorization and degradation was monitored using UV-Visible spectrophotometry, Fourier Transform Infrared (FT-IR) Spectroscopy and Gas Chromatography With Mass Spectrometry (GC-MS).

Results : *B. flabellifer* sprout peel sugar was supplemented as a macronutrient to support the growth of *Pseudomonas fluorescence* for the degradation of textile effluent. Higher decolorization efficiency (95%) within 7 days under aerobic condition at pH- 7.0 and temperature 35˚C was achieved.

Interpretation : The present study showed that the growth of *Pseudomonas fluorescence* was possible in tuber peel extracted sugars which was used as a carbon source. The bacteria grown in tuber peel extracted sugars was able to decolorize and degrade the textile effluent.

Key words : *Borassus flabellifer*, Degradation, Extracted sugars, *Pseudomonas fluorescence*



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Introduction

Currently research for suitable biomass to extract sugars as a carbon source for the growth of microorganisms is underway. Sugars present in the lignocellulosic biomass are tightly packed into crystalline cellulose polymers. These play a major role in the bioconversion of lignocellulosic biomass (Marcel Dekker, 2005; Peters, 2007; Lange,2007; Binder and Raines, 2009) which acts as substrates. Utilization of these raw materials yields reduced amount of sugars. It is also accompanied by other factors like presence of furfural derivatives which inhibits the growth and cost of hydrolysis. Pre-treatment methods to extract sugars are achieved by chemical and biological processes (Chaturvedi and Verma, 2013; Singh *et al.*, 2016). Most often chemical methods namely hydrolysis using concentrated or dilute acid are employed to delignify the biomass. The major advantage of acid hydrolysis is to increase the delignification rate faster than enzyme hydrolysis by penetrating the biomass to release sugars from the cellulosic framework. In some cases, ionic liquids were used to hydrolyse the biomass for bioconversion by the action of enzymes. (Dadi, 2006 ; Kilpelainen *et al.*, 2007 and Singh *et al.*, 2009). Ionic liquids produce lesser amount of glucose (Li and Zhao, 2007; Li *et al.*, 2008; Rinaldi *et al.,* 2008; Vanoye *et al.,* 2009 and Sievers *et al.*, 2009) whereas through hydrolysis of cellulosic biomass with concentrated acids and solvents high yield of glucose was obtained. On account of these factors lignocellulose-based biomass are selectively used for very few processes. To ensure that the utilization of hydrolyzed sugars does not inhibit microbial growth, the sugars derived from corn stover were supplemented with salts and the ethanologenic bacterium and yeast were grown (Binder *et al.*, 2010). Several agro wastes sugarcane bagasse, rice straw, bio-waste, peels of cassava, starch (cassava tubers) potato and banana (Arapoglou *et al.*, 2010; Benjamin *et al.*, 2014) have been explored for their reducing sugars to use as feedstock in biofuel production. The use of acid hydrolyzed potato peels for the extraction of total reducing sugars (Bhattacharyya *et al.*, 2013), cassava peel residues for its conversion into fermentable sugars (Souto *et al.*, 2017), pseudostem (Seenuvasan *et al.*, 2017) softwood (pine), hardwood (poplar, birch and beech), wheat straw and hemp (bast and harl) (Buzała *et al.*, 2017) and *Aloe vera* rinds (Sathya *et al.*, 2017) are also being bung exploited for raw material as an alternative to carbohydrates

Borassus flabellifer is a palm tree classified under Palmae family native to Africa. Palmyra sprout (known as Palmyra Tuber) is a sprout that grows on palmyra palms. The distribution of palmyra palms over the world is about 140 million. Tamil Nadu is a potential centre for the export of palm products. The growth and development of palm products industry is diversified in Tamil Nadu, India. Out of the estimated 8.59 cores of Palmyra in India, about 10 cores of Palmyra are grown in Tamil Nadu. The length of the shoot is between 12-15 cm before harvesting. Palmyra seed-shoot possess high starch content and are used in the preparation of palm spread and palm toffee (Churasiya *et al.*, 2014).The use of *Borassus flabellifer* peels for the analysis of glucose has been reported (Sathya *et al.*, 2017)

Sulphonated azo dyes are imposing a major threat to the aquatic life forms when discharged into the water stream. Proper treatment can reduce the risk of hazard to the live forms which are accompanied by biological treatments. The ability of bacterial strains that are able to degrade azo dyes under aerobic and anaerobic conditions has been extensively reported by many researchers. Bacterial strains that are able to degrade aromatic hydrocarbons have been repeatedly isolated, mainly from soil. These are usually Gram negative bacteria, most of them belong to the genus *Pseudomonas*.

Decolorization of Reactive blue 13 by *Pseudomonas* sp. under static conditions with efficiency of 83.2% has been reported (Lin *et al.*, 2010). The growth of *Pseudomonas fluorescence* on starch hydrolysate for polyhydroxybutyrate production is been reported (Aremu *et al.*, 2010). *Pseudomonas desmolyticum* NCIM2112 has been reported (Parte *et al.*, 2013) to degrade sulphonate aromatic compounds. The efficiency of *Pseudomonas aeruginosa* for rapid decolourization of azo dye, Cibacron Red was reported recently (Fetyan *et al.*, 2017). Palmyra sprout peels contain complex carbohydrate such as cellulose, hemicelluloses, lignin, etc. Pre treatment process such as acid hydrolysis followed by steam exposure can be used to degrade these complex carbohydrates to yield simple monosaccharides such as glucose. The extracted sugars are used as carbon source for the growth of *Pseudomonas fluorescence,* which are eminent sources for the degradation of pollutants. In view of the above, the present study focus on the use of Palmyra sprout peels for the extraction of fermentable sugars.

Materials and Methods

Chemicals required: Sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), activated carbon, 3,5 di-nitro salicylic acid, phenol, potassium sodium tartarate, anthrone, peptone, yeast extract, sodium chloride and glucose were purchased from Nice Chemicals, Mumbai. All the chemicals were of analytical grade and were used without any further purification. Palmyra sprout (*Borassus flabellifer*) was purchased from the local market (Chennai, India). The peels were removed carefully. It was washed thoroughly with distilled water, dried under shade for 20 days and the peels were chopped to few mm thickness.

Collection of textile effluent: Textile effluent was collected from textile dyeing unit located at Sangu Nagar, Erode at Tamil Nadu, India. The effluent from the textile unit was collected in a one litre airtight plastic container. It was filtered to remove suspended particles and the entire experiment was carried out under sterile condition.

Pretreatment and acid hydrolysis: To a 5 g of Palmyra sprout peels taken into a conical flask containing 200 ml of water, 5% of sulphuric acid was added. The flasks were covered with aluminium foil and incubated for 2 days at room temperature. The sample was autoclaved at 121˚C for 15 min. The flasks were allowed to cool and the pH was adjusted to 7.4 with NaOH. The sample was decolourized by passing it through No.1 Whatman filter paper containing activated carbon. The extracted glucose was determined by DNSA method (Miller, 1959) and the total carbohydrates were estimated by anthrone method (Trevelyan *et al.*, 1952).

Screening and biochemical characterization of bacteria for textile effluent degradation: The microorganism was isolated from soil by performing a serial dilution of 10^{-2} U. The dilution was spread to agar plate and incubated overnight at 30ºC. The colonies obtained were isolated and pure culture was maintained. Periodic sub culturing was done to maintain the stock culture. The bacterial isolate was characterized biochemically by Voges proskauer test methyl red test, indole test, and gelatine hydrolysis test (Mac Faddin, 1980).

Preparation of bacterial inoculum using extracted glucose: Standard inoculum was prepared by inoculating subculture of *Pseudomonas fluorescence* from the stock culture in a 250 ml conical flask containing 100 ml nutrient broth containing: 5 g l^{-1} Peptone; 1.5 g l^{-1} Yeast extract; 5 g l^{-1} NaCl and 1 g l^{-1} extracted glucose at 30 ºC for 3 days.

Analysis of metabolites: GC-MS analyses of the extracted metabolites were recorded by JEOL GCMATE II GC-MS with data system in a high resolution, double focussing instrument. An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC -5 column (25 µm I.D.25µm film thickness). A split injection was used for injecting sample and the split ratio was set to 10:1. The oven temperature was programmed to start at 35˚C, hold for 2 min, then ramp at 20 ºC per min to 260˚C and hold for 5mins. Helium carrier gas was set to 2 ml min^{-1} flow rate (constant flow mode).

The decolorization activity was calculated using the formulae Giwa *et al.* (2011):

$$\text{Decolorization activity (\%)} = \frac{(C_o - C_t)}{C_o} \times 100$$

Where, C_o = Initial absorbanc; C_t = Observed absorbance

Decolourization efficiency of textile effluent was recorded using a UV–Visible spectrophotometer UV1800 (Shimadzu, Japan) between 200 and 800 nm with water as blank. IR spectra of purified compounds were recorded on a Bruker series FT-IR spectrometer using KBr pellets.

Results and Discussion

Use of raw materials for any fermentation process add on to the costs of upstream processing. Researchers are exploring new and potential raw materials for the extraction of major carbon sources to be used in the media which will support the growth of microorganisms. Glucose yield from the pseudo stem by cellulose mediated hydrolysis was observed to be much higher when compared with the acid hydrolysis (Seenuvasan *et al.*, 2017). However, the fermentable sugars extracted from the Palmyra sprout peels in this study were identified to be a suitable raw material in favour of glucose by treatment with acid. The hydrolysed peels after neutralization were found to possess a sugar concentration of 1.27 mg ml^{-1} (Sathya *et al.*, 2017).

Pure culture isolated from soil was identified by performing biochemical characterization. The morphology and colour of the colonies were examined under microscope and was identified as Gram negative. The results of bacterial isolate analysed biochemically by methyl red test, Vogues Proskauer, gelatin hydrolysing test and indole test is shown in Table 1. The results revealed that the identified bacteria was *Pseudomonas fluorescence*.

The growth was monitored by periodic sampling for every 24 hr. The growth of *Pseudomonas fluorescence* concomitantly increased in the extracted glucose from the Palmyra sprout peels on comparison with the standard nutrient broth. The advantage of using *Pseudomonas fluorescence* is moderate supply of glucose which is enough for their maximum growth.

The increase in decolorization activity versus incubation time reveals that the decolorization of effluent proceeds by the metabolic activity of the microorganism (Kumar *et al.,* 2015, 2017; Vidhyadevi *et al.*, 2014).

The mass spectrum of textile effluent before degradation eluted out of the column at different time intervals is shown in Fig.

Table 1 : Biochemical characterization of isolated bacteria

Biochemical test	Identification	Results
Gram staining	Pink color, rod shaped	Gram negative
Methyl red	No change	Gram negative
Indole	No change	Gram Negative
Vogues Proskauer	Change pale yellow color	Gram Positive
Gelatin hydrolysing	Gelatin is liquefied	Gram Positive

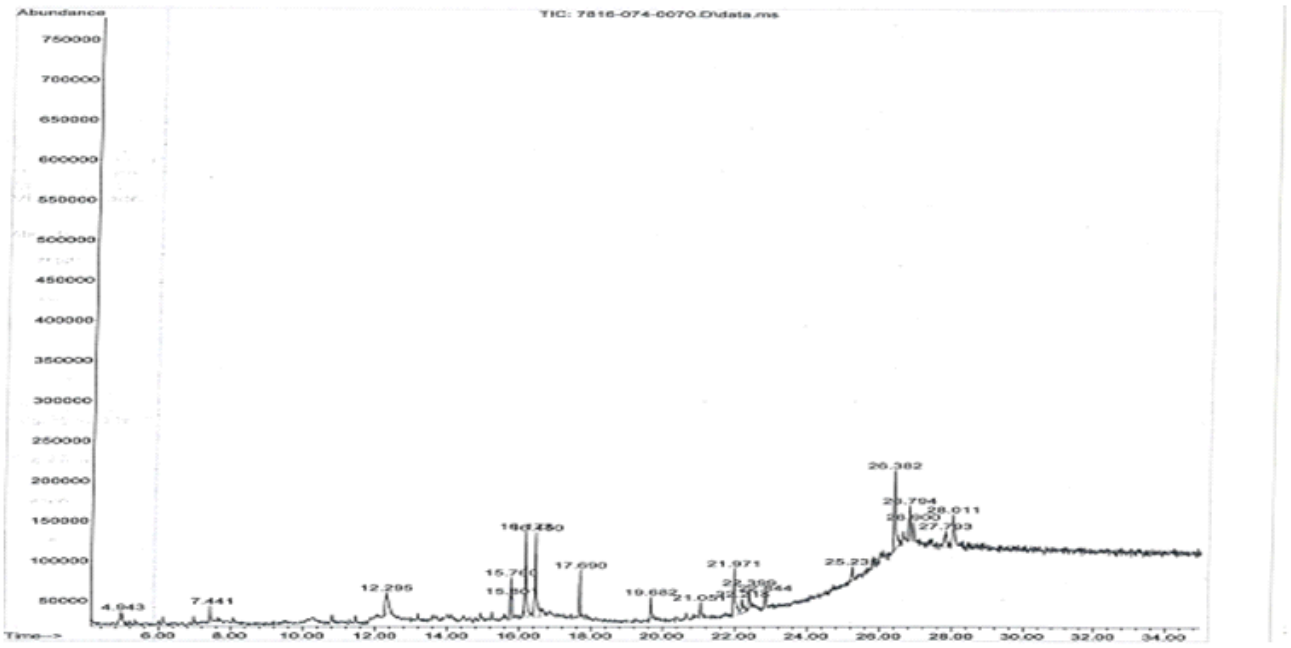

Fig. 1 : GC-MS analysis of textile effluent.

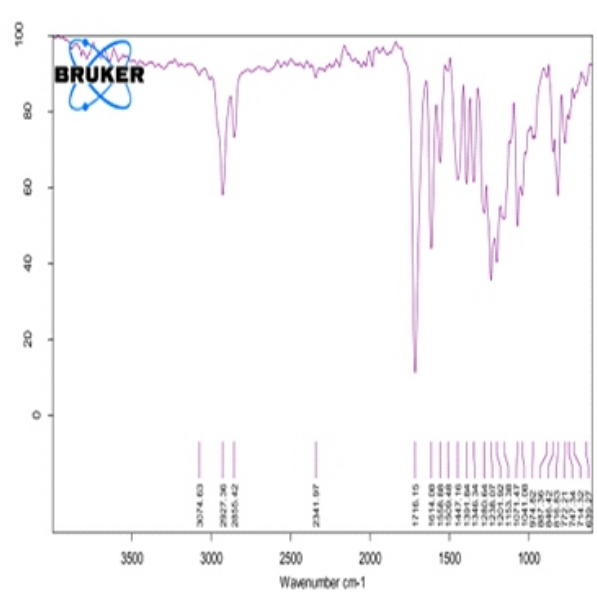

Fig. 2 : FT-IR spectra before effluent treatment.

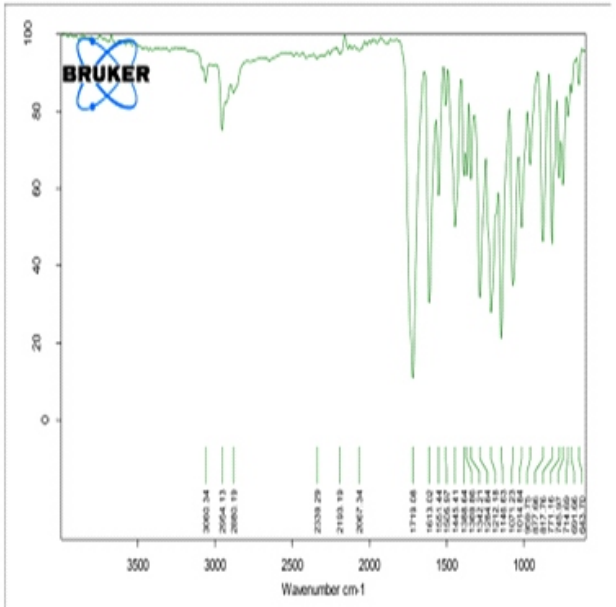

Fig. 3 : FT-IR spectra after effluent treatment.

1. It is evident that textile effluent contains toxic component like phthalic acid with retention time, Rt 16.17 min, methyl stearate Rt-17.69 min, tenamfetamine Rt-1.679 min, aspartic acid Rt-25.234 min, which cause diarrhea, hepatotoxicity and neurotoxicity. The most abundant metabolite is benzoquinoline with Rt of 28.014 min.

The FT-IR spectra of textile effluent before and after degradation are shown in Fig. 2 and 3. The peaks at 3074 cm^{-1},1614 cm^{-1},1346 cm^{-1} and 1280 cm^{-1} indicate the presence of C-H stretch (aromatic) which is benzoquinoline, N-H bend which is primary amine, CH rock (alkanes) and C-H stretch (aliphatic amine). Corroborate the previous study degradation of AR88 into aliphatic and tertiary amines by appearance of the peak at 1643 cm^{-1} for C=C stretch and peak at 1345 cm^{-1} for S=O stretch which indicated the presence of aromatic compounds (Kumar *et al*., 2017).

Whereas, the FT-IR spectra of the effluent metabolites is shown in Fig. 3. The peaks obtained at 3060 cm-1, 1613cm^{-1}, 1284 cm^{-1} and 1212 cm^{-1} after treatment showed deformation of aromatic compound benzoquinoline, alkane (Velayutham *et al.*, 2018) present in dye effluent. The results of spectra were identified according to Roeges and Baas,(1994) who offered a guide to the characteristics of the frequencies band in wave number to identify the functional groups according to the inter and intramolecular interactions created due to vibrational frequencies for the effluent.

To disclose the possible mechanism of effluent degradation, the product of biotransformation of textile effluent was analyzed using UV-visible spectrophotometer between 200 and 800 nm. On comparison with UV- visible absorption spectra before and after treatment, the peak slightly disappeared after treatment, thus revealing the occurrence of biodegradation (Kumar *et al.,* 2014).

Application of traditional effluent treatment requires high cost and continuous input of chemicals is uneconomical and causes environmental damage. Hence, economical and eco-friendly techniques using bacteria can be applied for effluent treatment. Biotreatment offers easy, cheaper and effective alternative for decolorization and degradation of textile effluent. Thus, the present study shows that the bacteria *Pseudomonas fluorescence* can be cultivated from extracted sugars and can be used as a good microbial source for the treatment of textile effluent. This study has established that bacteria are adaptive to the extracted sugars from Palmyra tuber peels in nature and can degrade the contaminants. It decolorized the textile effluent by degrading compound present in the effluent which cause health problems to humans.

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