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Treatment of rubber effluent from rubber processing plant with fungi

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SUMMARY. Rubber processing industry produces materials used for the manufacturing of rubber industrial products. The large volume of water is consumed and produces a huge amount of effluent which is later discharged into the waterways, thereby causing pollution that affects human health. The effluent was collected from discharge points of a rubber factory. Microbial analyses were carried out before and after pollution. During the incubation, microbial growth in culture tubes was determined using UV-Spectrophotometer by measuring absorbance at wavelength 600nm at 24 hours interval. Four fungi were isolated and identified from rubber effluents which include *Mucor mucedo* and *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum*. Individually, the selected fungi isolates were tested for its efficiency on the bioremediation of rubber processing effluent. The physicochemical properties reduction of the effluent such as BOD, COD, TS, TSS, TDS, phosphate and ammonia were observed after incubating for 7 days. Based on the data obtained in this study, it can be concluded that *Mucor mucedo* and *Aspergillus niger* can be used for bioremediation of rubber processing industry effluent with high efficiency.

Keywords: effluent, industry, indigenous, rubber.

Introduction

Rubber processing industry is one of the essential industries, which produce raw materials used for the manufacture of rubber industrial products such as conveyor belts, rubber rollers, automotive products like fan belts and radiator hoses, latex products which include rubber gloves and toys hygienic products and several kinds of adhesives. The main users of natural rubber are tire and footwear industries (Girish, 2014). The

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processing of these natural rubbers has several environmental impacts as pollution (Tekasakul and Tekasakul, 2006). The production process of rubber products from natural rubber needs enough volume of water for its operation producing large quantities of effluent (Leong *et al*., 2003; Rungruang and Babel, 2008). The disposal of these effluents into surface waters – wells, streams, lakes or even the sea without any treatment can give rise to a severe reduction of dissolved oxygen, thereby affecting the normal environment supporting the aquatic system (Mohammadi *et al*., 2010).

However, environmental damages generated from this industry could become big issues. Natural rubber processing sector consumes a great amount of water, energy and chemicals as well as other utilities. It also discharges enormous quantities of wastes and effluents (Leong *et al*., 2003). Wastewater is an unavoidable by-product of rubber processing: whatever processing procedures are used for preparing products from latex, there will always be an aqueous liquid as a by-product (Rungruang and Babel, 2008). When this wastewater finds their way into surface waters wells, streams, lakes or even the sea without any treatment, will certainly pollute those water bodies. The disposal of these effluents into water bodies can give rise to a serious reduction of dissolved oxygen, hence affecting the environment supporting the aquatic system (Mohammadi *et al*., 2010). The increasing universal concern on the environment demands that wastes should be properly managed in order to lessen and perhaps eradicate their potential harm to public health and the environment. Biodegradation is the process of utilizing indigenous microorganisms for the degradation of complex organic matter into simpler ones.

Rubber and effluents from rubber processing have been reported by researchers to support the growth of the microorganism (Atagana *et al*., 1999a; Bode *et al*., 2001; Cherian and Jayachandran, 2009). Owing to the need of biological treatment of rubber industry wastes and knowing the fact that various fungi and bacteria can grow and degrade the rubber industry wastes, the present study was aimed at isolating and characterizing indigenous fungi that can readily degrade the rubber wastes present in the effluents, with a view to developing an effective biological treatment.

Materials and methods

Sample collection

Rubber effluent samples were collected from discharge points of a rubber factory. For microbiological analysis, samples were collected in 500 ml sterile bottles. Clean plastic containers rinsed several times with the sample were used. The wastewater sample used for DO (dissolved oxygen) and BOD (biological oxygen demand) determinations were collected directly into dark DO bottles and were added some drops of manganous sulphate solution to fix the dissolved oxygen. Samples were collected by lowering the sterile bottle by means of a string into the tank and covered with the screw cap thereafter. The samples were stored at a temperature of 40C until required (usually between 24 and 48 hours).

Microbial analyses

Microbial analyses were carried out using the method of Cheesebrough (2000) and Cowan and Steele (1974) before and after pollution. 10 g of Soil sample was collected aseptically, labelled and store in ice packed plastic coolers and transported to the Environmental Biotechnology Sustainability Research laboratory, University of Benin, Nigeria where microbial analysis was carried out within 24 hours of sampling so as to maintain the stability of the sample without significant alteration in the microbial population.

Serial dilution was carried out by weighing 1 g of soil into 9 ml of sterile water contained in a 20 ml test tube and agitated to dislodge the microorganisms from the soil particles. From this initial dilution, a five-fold (10^{-5}) serial dilution was prepared.

Enumeration of heterotrophic fungi

The total heterotrophic fungi count was measured using the method of Taiwo and Oso (2004), by pour plating 1 ml of 10-3 dilution into Potato Dextrose Agar (PDA) supplemented with antibacterial agents (50 μg/ml of streptomycin and 30 μg/ml of penicillin) to inhibit the growth of bacterial contaminants. Fungal counts were observed and reported after 72 hours of incubation at room temperature (260C). Distinct fungal colonies were subculture repeatedly on freshly prepared Potato Dextrose Agar plates. Pure isolates of the microorganisms were maintained on agar slants as stock, which was preserved in the refrigerator for further use.

Characterization and identification of isolates

Distinct colonies of fungal isolates were characterized and identified based on their cultural and morphological features as described by Barnett and Hunter (1987). The characterizations were achieved through staining techniques-using lactophenol in cotton blue.

Acclimatization of isolates

The fungus was acclimatized by growing it in minimal organic salts medium amended with 10% of rubber processing industry effluent. The minimal medium used in degradation studies contained (mg/mL) KH_2PO_4 – 0.675; Na_2HPO_4 – 5.455; NH_4NO_3 – 0.25; $MgSO_4$ – 0.2; $Ca(NO_3)_2$ – 0.1; and 1 mL mineral solution (Table 1). The fungus was cultured in a 500-mL flask with the medium (100ml/flask) at 30^0C for 24 hours on a rotary shaker operating at 120 rpm. 1.0 mL of this was transferred aseptically to a second flask containing inorganic salts medium. After this solution became turbid, the culture was transferred to the third flask and incubated. This culture was used for biodegradation studies (Fig. 1).

Table 1.
Composition of minimal organic salt medium (Source: Gokul and Vijayan, 2015)

Composition	Quantity
KH_2PO_4	0.675 mg/L
Na_2HPO_4	5.455 mg/L
NH_4Cl	0.25 mg/L
$MgSO_4$	0.2 mg/L
$Ca(NO_3)_2$	0.1 mg/L

Biodegradation studies

Different parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), ammonia ($NH4^+$) and phosphate (PO43-) were assayed using standard protocols (APHA, 1995). The effluent was inoculated with 1% inoculum and incubated for 15 days and the estimation was done at the interval of 3 days.

At 24 hours interval during the incubation, microbial growth in culture tubes was determined spectrophotometrically by measuring absorbance at wavelength 600nm with a UV-visible spectrophotometer (Igiebor *et al*., 2017; Osarumwense and Igiebor, 2018).

Results and discussion

Effluents are an inevitable by-product of rubber processing, no matter the processing procedures employed for preparing products from latex (Rungruang and Babel, 2008). Asia and Akporhonor (2007) and Mohammadi *et al*. (2010) reported that effluents from rubber processing industries are very harmful and contain strong colour, a highly fluctuating pH, a large amount of suspended solids, high temperature, BOD and COD. Therefore, the treatment of rubber wastewater is a must before it is being disposed to natural water system (Atagana *et al*., 1999b; Iyagba *et al*., 2008). Most environmentally friendly process for effluent treatment is biodegradation utilizing indigenous microorganisms for the degradation of complex organic matter into simpler ones (Kumar *et al*., 2011). Bode *et al*. (2001), Cherian and Jayachandran (2009) revealed that the effluents from rubber processing have been known to support microbial growth; hence there is a need to isolate from the rubber effluents. The major purpose of the effluent treatment is to remove the suspended and soluble organic constituents measured as chemical oxygen demand (COD) or biochemical oxygen demand (BOD).

In the present study, a successful reduction of both BOD and COD of effluent from rubber processing industry was observed to a level adequate to make the effluent ready to be discharged into the environment, by treating with fungi inoculum. Percentage BOD and COD reduced significantly (within the range of 68 – 76 %) which was higher than the permissible limits. The reduction (64.09 mg/L) in Total Solids (TS) of rubber

effluent after treatment (Table 2) was within the permissible limit of 2100 mg/L (Gokul and Vijayan, 2015). The reduction of TS after treatment might be due to the use of suspended organics by microorganisms for their growth and development.

The reduction in BOD of rubber effluent after treatment (Table 2) showed a significant decrease in BOD values which could be attributed to the consumption of organic material by fungi as a source of food. The reduction in BOD after treatment can result in an instantaneous reduction of the microbial population. Although, high growth of fungi (microbes) had consumed the oxygen present in the treatment container. Furthermore, the continuous and excess aeration may have led to the reduction in BOD. The COD reduction (68.04%) could be due to the presence of high amounts of nutrients in the environment (Table 2), which may have favoured the growth of the isolates. The significant reduction in ammonia (248.97 mg/L – 118.63 mg/L) was observed after treatment, this could suggest that there was degradation of toxic solid components in the effluent by fungi.

Table 2.

Physio-chemical analysis of the effluent treatment

Parameters	Before treatment	After treatment	Percentage reduction
pH	3.36		
TDS (mg/L)	2797	1371	50.98 %
TSS (mg/L)	200	56	72.00 %
TS (mg/L)	2997	1076	64.09 %
Ammonia (mg/L)	248.97	118.63	52.35 %
Phosphate (mg/L)	15.53	8.76	42.31 %
BOD (mg/L)	380	89	76.58 %
COD (mg/L)	6940	2218	68.04 %

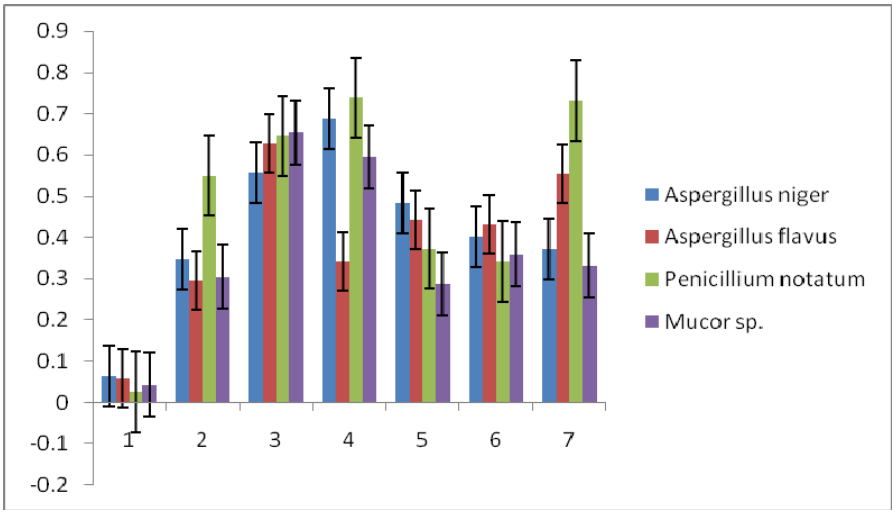

Figure 1. *In vitro* biodegradation studies of isolates

This study showed that the treatment of effluent with fungal isolates resulted in a successful reduction of BOD, COD, TDS, TSS, NH4+ and PO43 to a level of about 50 – 77%. This significant reduction is adequate to make the effluent set to be discharged into the environment. The treatment system that decreases or remove the level of $NH4^{+}$ and $PO4^{3-}$ compounds in the industrial effluent is termed to be highly effective (Ye *et al*., 1988). Microbial treatment (fungal) is also reported to reduce the levels of total suspended solids (TSS) and total dissolve solids (TDS) of industrial effluents (Arun *et al*., 2004; Monica *et al*., 2011), as observed in the present study.

Using Ward's method of cluster analysis, it was observed that *Mucor mucedo* and *Aspergillus niger* were most similar (Fig. 2) in enhancing remediative capacity of the rubber effluent followed by *Aspergillus flavus* than *Penicillium notatum*, which was a stand-alone. This suggests that *Mucor mucedo* and *Aspergillus niger* have the capability to treat rubber effluent.

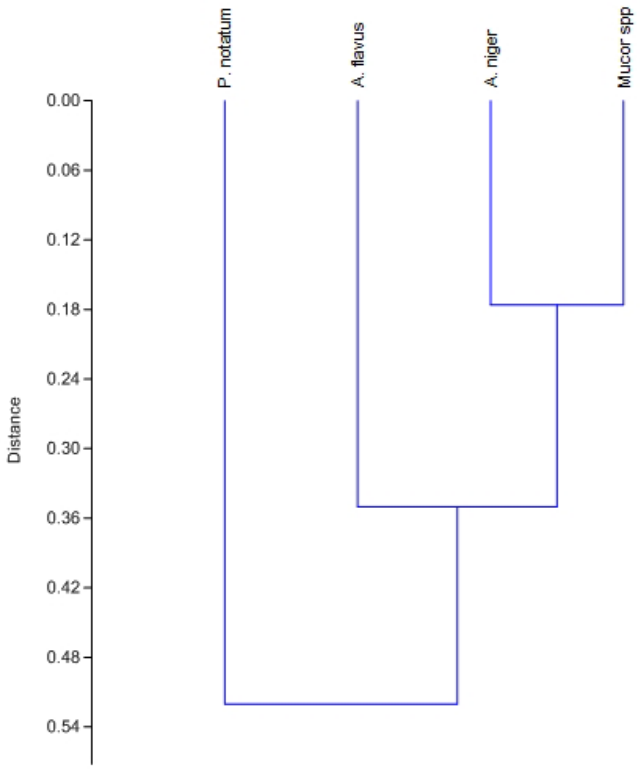

Figure 2. Dendrogram from cluster analyses of fungi isolates accumulated during the study

Conclusions

This study has successfully identified four indigenous fungi that could be used for the treatment of rubber processing effluent. The investigation further suggests that *Mucor mucedo* and *Aspergillus niger* isolated from rubber processing effluent could be employed for the biodegradation of rubber processing industry effluent. There is a need for molecular characterization for precise identification.

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