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ENHANCED BIODEGRADATION OF HIGH MOLECULAR WEIGHT PAHS USING YEAST CONSORTIA IMMOBILIZED ON MODIFIED BIOWASTE MATERIAL

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ABSTRACT

The degradation of high molecular weight (HMW) polycyclic aromatic hydrocarbons (PAHs) viz. benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene in soil was studied using yeast consortia immobilized on various conventional matrices and biowaste materials. Enhanced biodegradation was noted using yeast consortia immobilized in sawdust powder (SDP) which was chemically modified (M-SDP). The chemical modification destroyed the benzene rings in the cellulose and improved the porosity of SDP by decreasing the crystallinity of the biomass. The physicochemical properties of M-SDP were characterized with SEM, EDAX analysis, FTIR analysis, X-ray diffraction and TGA analysis. Application of M-SDP showed enhanced degradation of benzo[*a*]pyrene (82.5%) followed by perylene (75.1%) and benzo[*ghi*]perylene (63.4%) after 40 days of incubation. The results of kinetic study demonstrated that HMW PAHs degradation in soil fitted the first order kinetic model. It can be concluded that chemically modified SDP can serve as potential immobilization matrix for supporting pollutant-degrading yeast consortia which can be employed for effective degradation of HMW PAHs.

Keywords: Biodegradation, Chemical modification, HMW PAHs, Immoblization, Yeast consortium

INTRODUCTION

Pollution caused by polycyclic aromatic hydrocarbons (PAHs) is a matter of great concern throughout the world because of their toxic effects in the environment (**Deng *et al*., 2016; Alessandrello *et al*., 2017; Dutta *et al*., 2017**). PAHs are considered to be very harmful because of their recalcitrant, mutagenic and carcinogenic nature (**Bhattacharya *et al*., 2014; Cui *et al*., 2014; Chang *et al*., 2014; Ping *et al*., 2017**). The major strategies for PAH removal from environment include bioremediation (using microbes and plants), extraction with solvents, chemical oxidation or implementation of various physical methods such as electrokinetic remediation and thermal technologies (**Lau *et al*., 2014**).

Biodegradation is preferred for removal of pollutants from the environment because it represents an eco-friendly and economic process leading to the formation of less hazardous products (**Khan *et al*., 2015**). Microbial biodegradation has been proposed as a promising method compared to other methods for treating PAHs in the environment due to its simplicity, higher efficiency and cost effectiveness (**Yang *et al*., 2011; Patel *et al*., 2012**). However, the use of native biomass (such as bacteria, yeast, fungi and algae) for degradation of toxic compounds in freely suspended state is limited owing to their disadvantages such as small particle size, possible clogging and low mechanical strength of the biomass (**Godjevargova *et al*., 2004**). To overcome these limitations on the use of microorganisms for pollutant bioremediation, the microbial cells are often immobilized and modified which can increase the biodegradation rate by protecting the cells from sudden exposure to higher concentrations of PAHs (**Sarma and Pakshirajan, 2011**) and improving their tolerance against the toxic compound (**Baskaran and Nemati, 2006**).When immobilized microbial cells are used, the efficacy of biodegradation is often improved. Therefore, while using this strategy, the microbial biomass need to be immobilised in different support materials. It has been reported that immobilization of microorganisms enhances PAH degradation. It also facilitates the handling of microbial biomass and its application on the field (**Shimada *et al*., 2012; Mangwani *et al*., 2014**).

The use of biowaste materials or industrial products may be considered as support materials as carbon, nitrogen or energy sources for reducing the cost. This also provides an ecological alternative for waste management. There is report on application of biowaste material for the degradation of total petroleum hydrocarbon (TPH) using microbial consortia (**Nwankwegu *et al*., 2016**).

In the present study, yeast consortia have been immobilized on various conventional single matrices viz. agar, chitosan, CMC, sodium alginate and hybrid matrices viz. glycerol–sodium alginate (G-SA) and polyvinyl alcohol–sodium alginate (PVA-SA). To develop cost effective process, biowaste materials viz. peanut hull, wheat bran, rice husks and sawdust have also been used for immobilization of yeast consortia. Our previous work already demonstrated the potentiality of the yeast consortia (**Mandal *et al*., 2016; Mandal and Das, 2018**) for biodegradation of HMW PAHs. So far, there is no report regarding the application of immobilized yeast consortia using single and hybrid matrices as well as biowaste materials for the degradation of HMW PAHs from soil.

MATERIALS AND METHODS

Materials

Benzo[*a*]pyrene (BaP) (purity ≥ 96 %) and perylene (PRL) (purity ≥99 %) procured from Sigma-Aldrich, USA and benzo[*ghi*]perylene (BghiP) (99.3 %) was purchased from Supelco, USA respectively. Stock solutions of benzo[*a*]pyrene or perylene or benzo[*ghi*]perylene were prepared in chloroform and stored. All others chemicals used were of high quality, obtained from Hi-Media Laboratories (Mumbai, India), Merck (India) and SRL Pvt. Ltd. (Mumbai, India). Biowaste materials i.e. wheat bran was collected from local market, Vellore, Tamil Nadu, India whereas rice husk, peanut hull powder and sawdust powder were obtained from respective mills from Vellore.

Yeast consortia

The yeast consortia viz. YC01, YC02, YC03 and YC04, consisting of *Rhodotorula* sp. NS01, *Hanseniaspora opuntiae* NS02, *Debaryomyces hansenii* NS03 and *Hanseniaspora valbyensis* NS04, used in the present study were taken from our laboratory (**Mandal *et al*., 2016; Mandal and Das, 2018**).

Immobilization of yeast consortia in different matrices

Preliminary experiments were performed to screen the different immobilization matrices for efficient degradation of HMW PAHs. Yeast cells were cultured in

yeast broth for 48-72 h to obtain an optical density of 2. The yeast culture broth was centrifuged and washed 2-3 times with phosphate buffer (pH 7.0). The cells were resuspended in mineral medium (**Mandal *et al*., 2016 ; Mandal and Das, 2018**) for 48-72 h to obtain approximately 1×10^{10} cells ml^{-1}. The yeast consortia were immobilised in different matrices as reported by **Mulla *et al*., (2012)**. The viable cell counts were determined by the plate-count method (**Mulla *et al*., 2012**).

The preparation of beads using conventional immobilization matrices viz. agar, carboxyl methyl cellulose (CMC), chitosan and sodium alginate was carried out under sterilized condition following the method of **Chandran and Das (2011)** and **Lakshmi and Das (2013)** with minor modifications.

Agar

Agar solution (2%) was prepared and melted by heating under sterilized condition. 100 ml of agar solution was cooled down to the temperature below 40 ˚C and mixed with yeasts suspension (1×10^{10} cells ml^{-1}) under sterilized condition to achieve above 4 g wet weight l^{-1} a cell concentration. Yeast suspension and mixture of agar solution was poured gently into sterilized petri plates coated with thin layer of refined sunflower oil until it solidified. After solidification, immobilized agar blocks were cut into equal size of around 2 mm cubes.

Carboxyl methyl cellulose (CMC)

Yeast suspension (1×10^{10} cells ml^{-1}) was mixed thoroughly with 100 ml of CMC solution (2%). The mixture of CMC-yeast suspension cells was injected using an injector to form beads drop-wise to $FeCl_3$ solution (0.05 M). To enhance the mechanical stabilities, yeast immobilized beads were cured for 1 h in the solution of $FeCl_3$.

Chitosan

Yeast cells (1×10^{10} cells ml^{-1}) were transferred to sterilized solution (30 ml) of chitosan (2%) prepared in acetic acid (1%) having pH value of 7.5. For beads formation and coagulation, the mixture was injected drop-wise through a plastic tip (1.0 ml) into solution of NaOH (8%). After interval of 0.5 h, the immobilized beads were taken out from the solution and washed two times in 200 ml of sterilized water for 15 min under agitation for inoculation.

Sodium-alginate

Yeast suspension (1×10^{10} cells ml^{-1}) was mixed with 100 ml of sodium alginate solution (2%). The yeast–sodium alginate mixture was dropped into $CaCl_2$ (0.2 M) gently. To enhance their mechanical stabilities, immobilized beads were cured for 2 h in $CaCl_2$ (0.2 M).

Hybrid matrices

Sodium alginate (10 g l^{-1}) and PVA (10 g l^{-1}) were mixed into sterilized distilled water at 80 °C to prepare hybrid matrix. Initially mixed solution of PVA and sodium alginate (PVA-SA) was cooled down to room temperature and mixed properly to get a suspended homogenous mixture. Aliquot of yeast suspension (5 ml) was added to the PVA-SA solution mixture. Ratio of the final matrix of PVA and SA as 1:1 was maintained. The PVA-SA mixtures were exuded in $CaCl_2$ (0.2 M) to form 0.26 ±0.2 cm diameter beads. The immobilized beads were washed twice with sterilized saline solution before used for experimental study. Similar method was followed for the preparation of glycerol-sodium alginate (G-SA) mixture beads at same ratio (1:1) as mentioned above .

Biowaste materials

Biowaste materials viz., peanut hull, rice husk, sawdust carrier, and wheat bran materials each 0.3 g was sterilized for 15-20 min. Each vial containing the sterilized biowaste material was inoculated with 16 mg dry wt ml^{-1} of yeast cell suspension (1 ml) in phosphate buffer solution (10 mM) prepared from culture broth of yeast extract peptone dextrose (YEPD) grown for 24 hr. The vials were incubated for a period of 4 h and washed by resuspending the carrier cultures in 2 ml of mineral medium **(Mandal *et al*., 2016 ; Mandal and Das, 2018**) and the supernatants were discarded. Subsequently, the vials containing the carrier cultures were incubated at 35 °C for various periods (4, 24 and 96 h) to determine the optimum incubation period necessary for the development of a well-established (immobilized) culture on the carriers. After each incubation period, replicate cultures were withdrawn and dried in a sterile hood in a stream of dry air at room temperature for 4 days. The viability of dried carrier cultures was tested at different time intervals over a period of 10 weeks by total plate count technique.

Screening of different matrices for HMW PAHs degradation in soil

The soil was collected from the nursery in VIT University, Vellore which had no record of HMW PAHs contamination previously. The pH of soil sample was 7.9 and classified as loamy clay containing 2.4 % organic carbon, 6 % sand, 25 % silt and 56% clay. The collected soil was air dried, sieved and amended with biomanure. BaP, PRL and BghiP were dissolved in chloroform to a final concentration of 50 mg kg^{-1}, 50 mg kg^{-1} and 40 mg kg^{-1} respectively and added to the soil and mixed well. After the solvent was evaporated, the spiked soils were used for experiments.

To screen the best matrix for immobilization, yeast consortia immobilized on various matrices were kept in 250 ml conical flask containing 200 g PAH spiked soil. 2 g of the soil sample was extracted three times by ultrasonic treatment for 20 min with an Ultrasonic Disrupter containing 30 ml mixture of hexane-acetone (1:1) followed by centrifugation at 3000 rpm for 10 min to separate the supernatant from the soil. The supernatant was collected in a 100 ml round bottom flask (**Sun *et al*., 1998**). The extract was concentrated using rotary evaporator and re-dissolved in hexane before analysis. Residual HMW PAHs were quantified by gas chromatography (**Zhao *et al*., 2014**). Concentration of residual HMW PAHs after degradation was calculated comparing the peak area of the sample and control.

Modification and characterization of biowaste material

The biowaste material, sawdust powder was chemically modified (M-SDP) and characterized. M SDP was prepared by soaking SDP in sodium hydroxide and hydrogen peroxide solution (1% respectively, m/m) with stirring at room temperature for 14 h. SDP and M-SDP were used as the immobilization carriers of yeast consortium. The samples of the carrier were then put into polyethylene bags resistant to high voltage and high temperature and sterilized by autoclaving at 121 °C, 1×10^{5} pa for 20 min before use. The immobilization of the microbes was carried out by mixing the carrier with the free inoculum at room temperature (**Labana *et al*., 2005**).

For characterization, samples of SDP and M–SDP were dissolved in ethyl acetate and mixed with KBr to form the pellets. Infrared spectroscopy was done with an IR affinity-1 FT-IR spectrophotometer (Shimadzu). The scanning wavelength range was kept from 4,000 to 400 cm^{-1} and the spectral resolution was 4 cm^{-1}.

The surface morphology of SDP and M-SDP was analyzed using scanning electron microscopy (SEM) (Stereo Scan LEO, Model-400). EDAX analyses was conducted using Noran System Six model Energy Dispersive X-Ray Microanalysis System (Thermo Electron Corporation, Japan) attached to SEM. Accelerating voltage was kept constant at 15 kV to facilitate the emission of secondary X-rays.

The crystallinity of SDP and M-SDP was characterized using X-ray diffraction (XRD) patterns (Bruker D8 Advance diffractometer). The sample was exposed to Cu-Kα radiation in the 2θ range of 20°-80° with a scanning rate of 4 min and step size of 0.02. Thermogravimetric analysis of the photocatalysts was carried out under high purity helium supplied at a purge gas flow rate of 0–1000 ml min^{-1} (Diamond TG/DTA, Perkin Elmer, USA). Samples were subjected to 10 °C min^{-1} heating rate and characterized between 25 and 800 °C.

Biodegradation of HMW PAHs by immobilized yeast consortium using M-SDP

Degradation of HMW PAHs in soil by yeast consortium (YC01, YC02 and YC04) immobilized in SDP and M-SDP were conducted in 250ml conical flasks containing 200 g of soil supplemented with 50 mg kg^{-1} (BaP and PRL) and 40 (BghiP) as sole carbon and energy source. The yeast consortium immobilized in SDP and M-SDP (0.5 g) was added to the flasks to the cell concentration of 1×10^{10} cells ml^{-1}. Flasks were then incubated at 30 °C. HMW PAHs in free cells (yeast consortia without immobilization) and immobilized treatments were extracted following the method already mentioned.

Kinetics study

The experimental data of the degradation kinetics of HMW PAHs were fitted with zero (**Wang *et al*., 2002**) and first order (**Agarry *et al*., 2013**) kinetic models.

Statistical analysis

Mean of three replications was considered as the final reading for all the analysis. Data were analyzed using analysis of variance (ANOVA) using Prism6 software (Graph Pad Inc.) to determine the significance of difference between the treatments on HMW PAHs degradation in the experiments. The statistical significance in this analysis was defined at $p < 0.05$.

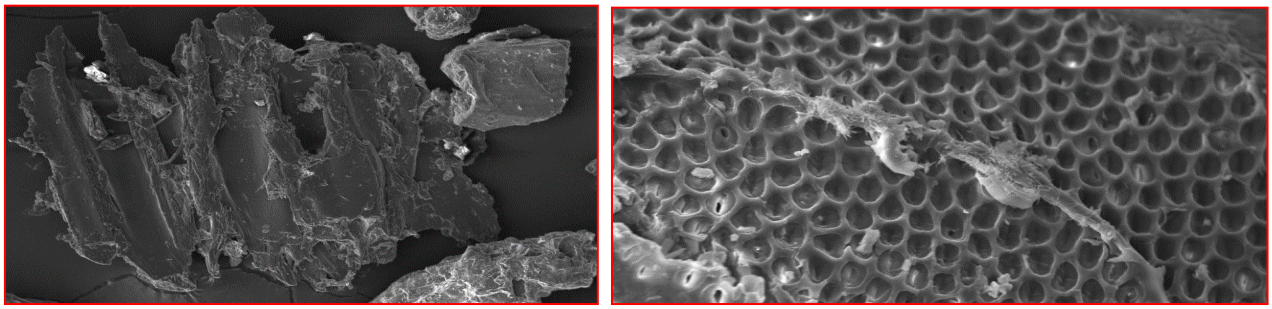
RESULTS AND DISCUSSION

Screening of different matrices for HMW PAHs degradation using immobilized yeast consortia

The viability of the immobilized dried yeast consortia in different matrices was checked for a period of 10 weeks at room temperature. Degradation experiments by immobilized yeast consortia in different matrices were performed in batch experiments shown in Table 1. Among the single matrices viz. sodium alginate, agar, CMC and chitosan used in the study, it was found that immobilization of yeast consortia in sodium alginate showed best performance which degraded BaP (71.6%) using YC01, PRL (64.9 %) using YC02 and BghiP (53.2%) using YC04. Best performance of sodium alginate as single matrix for immobilization of yeast cells was reported by Lakshmi and Das (2013). In case of hybrid matrix, PVA-alginate immobilied yeast consortium showed maximum degradation of BaP (75.8 %) by YC01, PRL (68.5 %) by YC02 and BghiP (55.3%) by YC04. In PVA-alginate hybrid matrix, alginate increases the surface properties and reduces agglomeration whereas PVA contributes high crosslinking capacity and strength (**Sasaki *et al*., 2007**). Some workers have already reported PVA-alginate hybrid matrix as a promising carrier for immobilization of microorganisms (**El-Naas *et al*., 2013; Selvi *et al*., 2015**).

Table 1 HMW PAHs degradation potential of free cells and immobilised yeast consortia in different matrices after 40 days

Different matrices	Benzo[*a*]pyrene degradation (%)			
	YC01	YC02	YC03	YC04
Free cells	64.2±0.05	59.9±0.02	63.1±0.10	62.6±0.08
Immobilized cells				
Immobilized matrices				
Single matrix				
Agar	66.3±0.03	60.5±0.04	64.8±0.05	63.4±0.07
Chitosan	67.8±0.20	62.3±0.01	65.2±0.03	64.7±0.02
CMC	70.1±0.07	65.9±0.06	68.3±0.09	67.8±0.04
Sodium alginate	71.6±0.06	66.2±0.03	69.5±0.01	68.1±0.08
Hybrid matrix				
G-SA	73.1±0.06	68.4±0.15	71.7±0.09	70.5±0.04
PVA-A	75.8±0.20	69.9±0.05	73.1±0.07	72.6±0.02
Biowaste materials				
Rice husk	76.3±0.11			
Wheat bran	71.8±0.07			
Peanut hull	74.6±0.02			
Sawdust	78.1±0.04			
Different matrices	Perylene degradation (%)			
Free cells	55.7±0.07	58.3±0.03	51.6±0.01	50.8±0.05
Immobilized cells				
Immobilized matrices				
Single matrix				
Agar	56.8±0.09	59.1±0.02	52.9±0.08	51.5±0.10
Chitosan	57.5±0.30	60.7±0.07	53.1±0.04	52.6±0.06
CMC	59.2±0.15	63.2±0.11	56.4±0.09	54.5±0.02
Sodium alginate	60.1±0.03	64.9±0.01	57.6±0.07	56.8±0.21
Hybrid matrix				
G-SA	63.7±0.05	66.2±0.80	59.6±0.03	58.4±0.01
PVA-A	65.3±0.08	68.5±0.12	61.4±0.09	60.8±0.07
Biowaste materials				
Rice husk		67.9±0.01		
Wheat bran		64.7±0.04		
Peanut hull		62.3±0.08		
Sawdust		72.0±0.03		
Different matrices	Benzo[*ghi*]perylene degradation (%)			
Free cells	48.5±0.06	45.1±0.04	47.3±0.13	49.1±0.09
Immobilized cells				
Immobilized matrices				
Single matrix				
Agar	49.3±0.08	46.4±0.02	48.7±0.07	50.4±0.10
Chitosan	50.6±0.25	47.5±0.07	49.1±0.05	51.8±0.02
CMC	51.4±0.04	48.3±0.09	50.8±0.10	52.4±0.31
Sodium alginate	52.4±0.10	49.8±0.02	51.1±0.06	53.2±0.09
Hybrid matrix				
G-A	53.2±0.20	50.5±0.05	52.9±0.13	54.7±0.01
PVA-SA	54.7±0.04	51.1±0.17	53.2±0.06	55.3±0.08
Biowaste materials				
Rice husk				58.1±0.06
Wheat bran				57.5±0.07
Peanut hull				56.2±0.02
Sawdust				59.5±0.05



Sawdust powder (SDP) Modified sawdust powder (M-SDP)

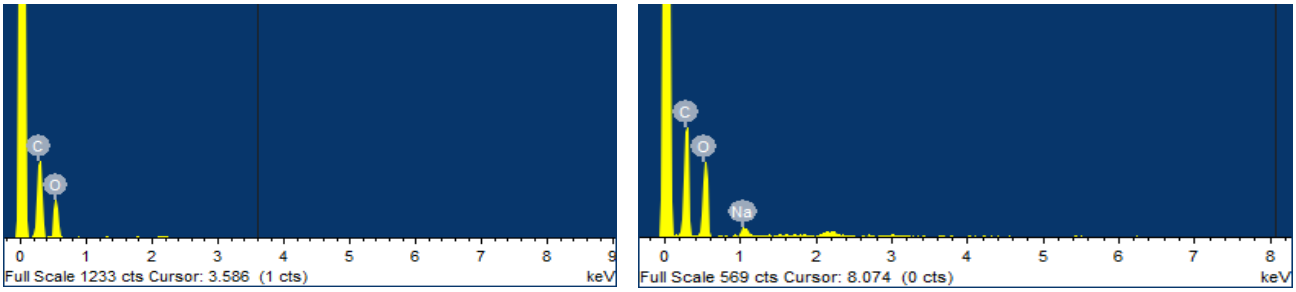
Figure 1 Scanning electronic microscope (SEM) analysis of sawdust powder and modified sawdust powder

Based on the screening of single and hybrid matrices, yeast consortium YC01 for BaP, YC02 for PRL and YC04 for BghiP were selected for further immobilization study using biowaste materials viz. rice husk, wheat bran, peanut hull and sawdust. The use of sawdust as carrier material greatly increased the biodegradation of BaP (78.1%), PRL (72%) and BghiP (59.5%) within 40 days (Table 1). Sawdust is generated wastes by wood workers usually awaiting disposal either in municipal dumpsites or by incineration. The application of sawdust has already been reported as an eco-friendly and cost effective remediation material for bioremediation of environmental pollutants (**Mane and Babu, 2011**). Therefore, based on the screening of biowaste materials, sawdust powder (SDP) was chemically modified for the improvement of HMW PAHs biodegradation efficiency.

Characterization of SDP and modified-SDP

SEM and EDAX analysis

Scanning electronic microscope (SEM) micrographs of the support materials SDP and M-SDP are shown in figure 1. The photograph showed that the M-SDP had greater porosity looks like beehive than SDP which allowed for better adsorption of yeast consortium cells onto the carrier.



Sawdust powder (SDP) Modified sawdust powder (M-SDP)

Figure 2 EDAX analysis of sawdust powder and modified sawdust powder

The elemental analysis of SDP before and after chemical modification was performed using energy dispersive Xray (EDX) analysis (Figure 2). The EDX spectrum of SDP before modification indicated the presence of C and O as naturally present on the sawdust powder. The peak of Na was noted on M-SDP which confirmed the modification of SDP.

FTIR analyis

The results of FTIR analysis of SDP sample before and after modification are shown in figure 3 which reflect the major changes of the chemical components of SDP during treatment. The peaks of SDP before modification are assigned as follows: 3332.99 cm^{-1} for –OH stretch in alchols and phenols; 2902.87 cm^{-1} for CH antisym and sym stretching in aliphatic compounds; 1730.15 cm^{-1} for C=O sym stretch in anhydrides ; 1600.92 cm^{-1} for antisym stretch in carboxylic acid salts; 1506.41 cm^{-1} for ring stretch and sharp band in benzene ring; 1446.61 cm^{-1} for CH_3 deformation in aliphatic groups; 1261.45 cm^{-1} for C-O stretch in epoxides; 1157.29, 1107.14 and 1028.06 cm^{-1} for C-O stretch in C-OH (alcohols); 894.97 cm^{-1} for CH_2 out of plane wag in vinylidenes; 653.87 cm^{-1} for C-C-CHO bending in aldehydes; 503.42 cm^{-1} for C-O-C bend in ethers. Peaks of M-SDP (after modification of SDP) are assigned as follows: 3350.35 cm^{-1} for OH stretch in alcohols and phenols; 2885.51 cm^{-1} for CH antisym and sym stretching in aliphatic compounds; 1598.99 cm^{-1} for C=O stretch; enol form in β-diketones; 1105.21 cm^{-1} for C-O stretch in C-OH (alcohols); 655.80 cm^{-1} for C-C-CHO bending in aldehydes; 561.29 cm^{-1} for in-plane and out of plane ring deformations in benzene derivatives. Therefore, it is found that peaks in FTIR spectra are significantly modified which are typically associated with O-H, C-H, C=O and C-O bonds indicating that SDP is strengthened after modification with hydrogen peroxide and sodium hydroxide solution.

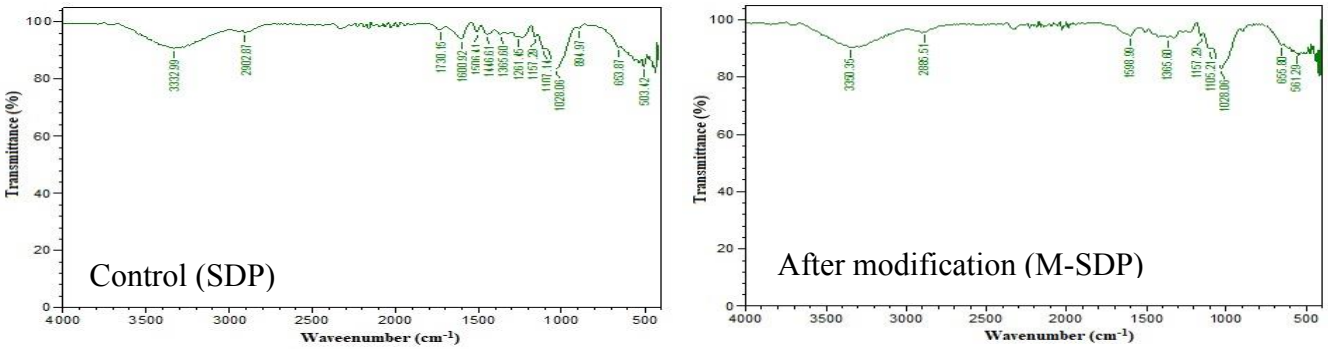


Figure 3 FT-IR spectra of sawdust powder and modified sawdust powder

XRD analysis

X-ray diffraction patterns of SDP and M-SDP were determined in the range of 20-90 θ and the results are presented in figure 4. The XRD pattern of both SDP and M-SDP exhibited a well defined peak thereby indicating their purity. The XRD pattern of SDP showed peaks at 2θ values of 15.02, 22.16 and 34.15 which correspond to the 120, 110 and 221 planes respectively. For the M-SDP, the peaks obtained were at 2θ values of 15.59, 22.47 and 34.96 which correspond to the 101, 120 and 122 planes respectively. Change in 2θ values indicates that the SDP was chemically modified.

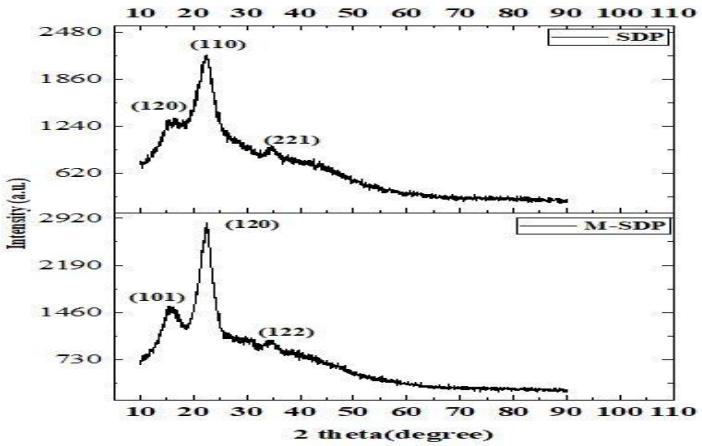


Figure 4 XRD patteerns of sawdust powder and modified sawdust powder

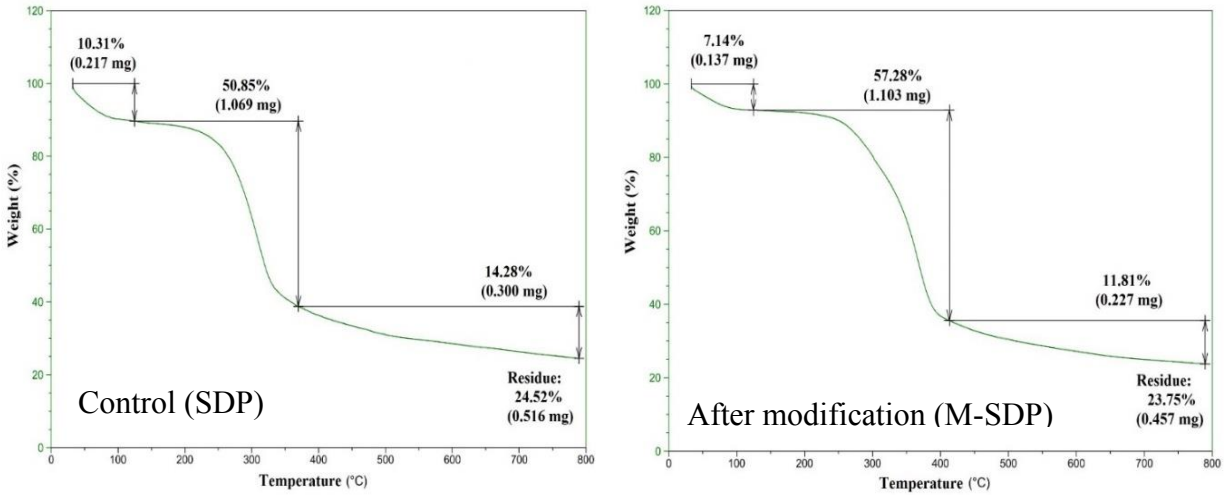


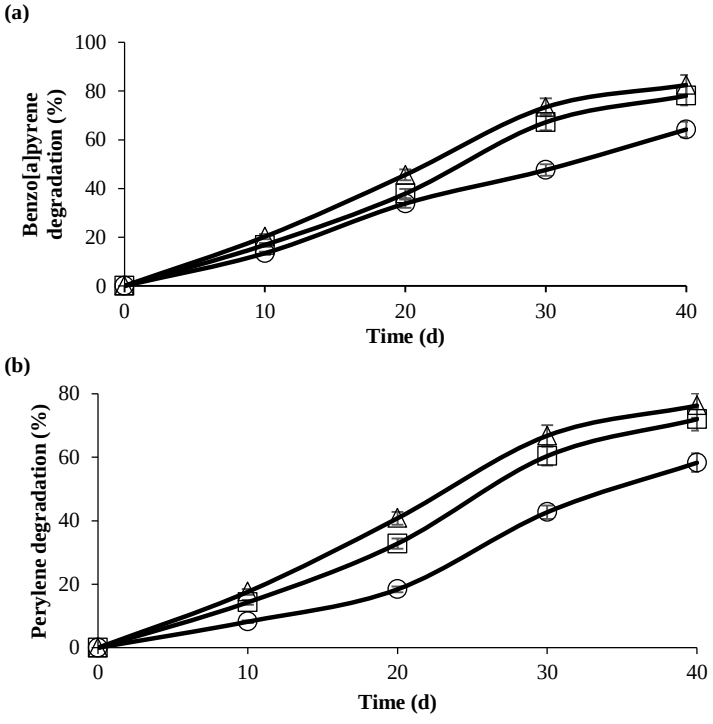
Figure 5 TGA analysis of sawdust powder and modified sawdust powder

TGA analysis

TGA measurement was used to study the thermal stability of the materials which measures the change of sample mass (weight loss associated with dehydration, decomposition and oxidation of sample) as a function of temperature (**Ahmed *et al*., 2013**). TGA of the SDP and M-SDP were shown in Figure 5. The plot of TGA curve is the conversion of TGA signal to percent weight loss against the sample temperature (°C). Thermogram of SDP revealed that the loss of weight occurred initially with ~10.31% (SDP) and ~7.14% (M-SDP) in the temperature between 32 °C to 100 °C (around) which may be associated with the loss of moisture contents (Ismail et al. 2010; Ahmed et al. 2013). The weight of bio-waste materials dramatically reduced above 400 °C for the both the cases. As the temperature is increased further, the residue of M-SDP was 0.457 mg (23.75%) and residue of SDP was 0.516 mg (24.52%). The results inicate that M-SDP is more thermostable than SDP.

Biodegradation of HMW PAHs using modified SDP

The degradation of HMW PAHs viz. BaP, PRL and BghiP at concentrations of 50 mg kg^{-1}, 50 mg kg^{-1} and 40 mg kg^{-1} respectively by immobilized yeast consortia at 30 °C using SDP and M-SDP are shown in Figure 6(a-c). For benzo[*a*]pyrene, the degradation with M-SDP, SDP using immobilized yeast consortium and free cells were found to be 82.5 %, 78.1 % and 64.2% respectively after 40 days (Figure 6a).



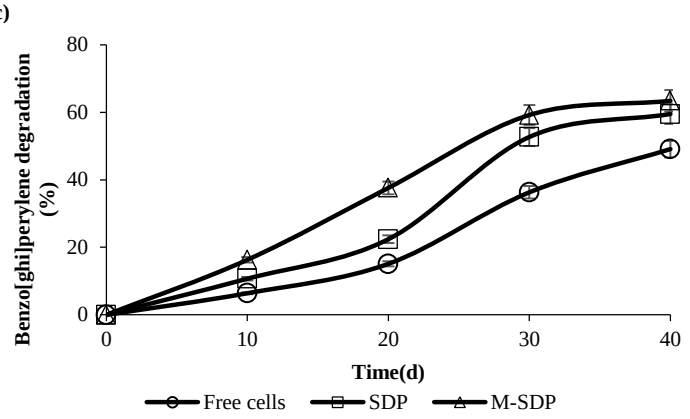


Figure 6 Degradation of HMW PAHs **a** BaP, **b** PRL and **c** BghiP using free cells(o), immobilised yeast consortium in sawdust powder(□) and immobilised yeast consortium in modified sawdust powder (Δ).

In case of perylene, the degradation with M-SDP, SDP using immobilized yeast consortium and free cells were noted as 75.1 %, 72.0 % and 58.3% respectively (Figure 6b). The degradation of benzo[*ghi*]perylene with M-SDP, SDP using immobilized yeast consortium and free cells was noted to be 63.4 %, 59.5 % and 49.1% at end of 40 days which was significantly lower than BaP and PRL (Figure 6c). Based on the results, it can be seen that significant increase in degradation of HMW PAHs was noted using M-SDP after a period of 40 days. The increase in degradation may be due to the improved porosity of the sawdust caused by chemical modifications of the sawdust surfaces. The increased porosity enhanced the oxygen dispersion and increased the adsorption and growth of yeast consortia resulting in increased microbial activity and biodegradation (**Xu and Lu, 2010**). Therefore, application of chemically modified sawdust can serve as efficient biocarrier material for immobilization of microbial consortium and can be taken as low-cost strategy for remediation of HMW PAHs.

Kinetics study

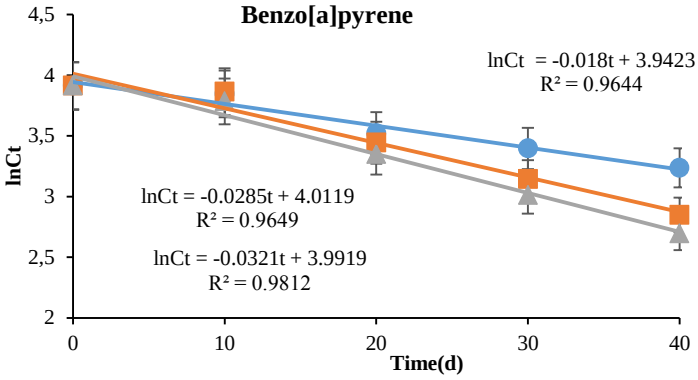
The kinetic data on degradation of benzo[*a*]pyrene (50 mg kg^{-1}), perylene (50 mg kg^{-1}) and benzo[*ghi*]perylene (40 mg kg^{-1}) were fitted with the first and zero mathematical kinetic models (Figure 7a-c). The results showed that the degradation kinetics of benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene by yeast consortium, YC01 YC02 and YC04 can be described well by first order reaction kinetics with respect to the concentration of benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene. The highest regression coefficient (R^2) values of (0.981), (0.976) and (0.967) in first order kinetic model indicated that the degradation kinetics of benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene followed first order reactions in case of immobilised yeast consortium on M-SDP (Table 2). This implied that the removal of benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene by yeast consortium were time dependent process and degradation rate was directly proportional to substrate concentration (**Deng *et al*., 2012; Jin *et al*., 2017**).

The calculated degradation rate constant (K) of benzo[*a*]pyrene is 0.032 d^{-1} and the theoretical half-life of benzo[*a*]pyrene is 21.656 days; calculated degradation rate constant (K) of perylene is 0.033 d^{-1} and the theoretical half-life of perylene is 21.0 days whereas calculated degradation rate constant (K) of benzo[*ghi*]perylene is 0.030 d^{-1} and the theoretical half-life of benzo[*ghi*]perylene

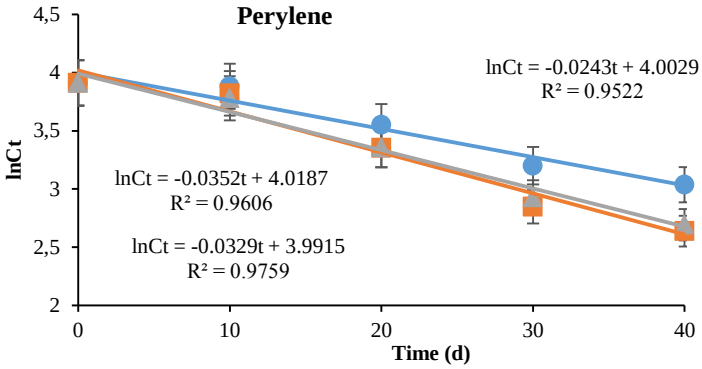
is 23.1 days in case of immobilised yeast consortium on M-SDP. It was noted that the higher the biodegradation rate constant, faster is the rate of biodegradation and consequently, the half-life times are lower.

Table 3 shows the biodegradation of HMW PAHs after 40 days using free cells and yeast consortium immobilized on chemically modified sawdust. Enhancement of degradation was found to be 18.3%, 16.8% and 14.3% in case of benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene.

a)



(b)



(c)

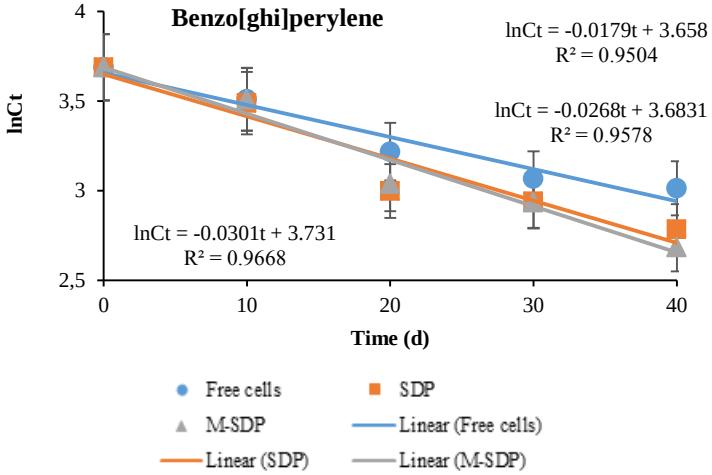


Figure 7 Plot of ln Ct vs reaction time (days) for different types of HMW PAHs **a** BaP, **b** PRL and **c** BghiP. The biodegradation rate constant for each HMW PAHs is computed from the slope of the line. The degradation of HMW PAHs by yeast consortium follows the first order kinetics.

Table 2 Kinetics parameters for the degradation of HMW PAHs by immobilised yeast consortium in modified sawdust powder.

Kinetics Model	Parameters	Free cells	SDP	M-SDP
		Benzo[*a*]pyrene		
Zero order	Regression equation	C_t= -0.660t+50.39	C_t= -0.899t+51.87	C_t= -0.941t+50.34
	K_0 (day)	0.660	0.899	0.941
	$T_{1/2} = C_0/2K_0$	37.879	27.807	25.765
	R^2	0.949	0.956	0.971
First order	Regression equation	lnC_t = -0.018t+3.942	lnC_t = -0.029t+4.012	lnC_t= -0.032t+3.992
	K_1 (day)	0.018	0.029	0.032
	$T_{1/2} = \ln 2/K_1$	38.508	23.890	21.656
	R^2	0.964	0.965	0.981
		Perylene		
Zero order	Regression equation	C_t= -0.822t+52.21	C_t= -1.004t+51.188	C_t= -0.955t+50.3
	K_0 (day)	0.822	1.004	0.955
	$T_{1/2} = C_0/2K_0$	30.414	24.9	26.316
	R^2	0.948	0.951	0.967
First order	Regression equation	lnC_t = -0.024t+4.003	lnC_t = -0.035t+4.019	lnC_t = -0.033t+3.992
	K_1 (day)	0.024	0.035	0.033
	$T_{1/2} = \ln 2/K_1$	28.875	19.80	21.0
	R^2	0.952	0.961	0.976
		Benzo[*ghi*]perylene		
Zero order	Regression equation	C_t= -0.512t+38.296	C_t= -0.622t+38.336	C_t= -0.709t+39.196
	K_0 (day)	0.512	0.622	0.709
	$T_{1/2} = C_0/2K_0$	39.063	30.211	28.209
	R^2	0.926	0.929	0.959
First order	Regression equation	lnC_t = -0.018t+3.658	lnC_t = -0.027t+3.683	lnC_t = -0.030t+3.731
	K_1 (day)	0.018	0.027	0.030
	$T_{1/2} = \ln 2/K_1$	38.508	25.667	23.1
	R^2	0.950	0.958	0.967

Table 3 Summary of biodegradation of HMW PAHs using free cells and yeast consortium immobilized on chemically modified sawdust

	BaP degradation using YC01	PRL degradation using YC02	BghiP degradation using YC04
Free cells	64.2 %	58.3 %	49.1 %
SDP	78.1 %	72.0 %	59.5 %
M-SDP	82.5 %	75.1 %	63.4 %

CONCLUSIONS

The present study demonstrated that remarkable enhancement in degradation of HMW PAHs was noted using yeast consortium immobilized on chemically modified sawdust (M-SDP). Experimental results also showed that modification of SDP significantly improved the biodegradation of (BaP and PRL) at a high concentration of 50 mg kg^{-1} and(BghiP) at concentration of 40 mg kg^{-1}. The SEM photograph showed that modification of SDP improved the porosity of SDP and yeast consortium cells were firmly immobilized in M-SDP. Other instrumental analysis viz. EDAX, FTIR , TGA and XRD confirmed the chemical modification of SDP. Therefore, it can be concluded that M-SDP is a good choice for the enhancement of degradation of HMW PAHs through the use of immobilized yeast consortium. To the best of our knowledge, this is the first report in which modified biowaste material (sawdust) have been effectively used for the enhanced degradation of high molecular weight PAHs using immobilized yeast consortia.

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Conflict of interest The authors declare that they have no conflict of interest.

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