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Biodegradation of perylene and benzo[ghi] perylene (5-6 rings) using yeast consortium : Kinetic study, enzyme analysis and degradation pathway

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Abstract

Aim : The present work aims to demonstrate the ability of yeast consortium for degradation of perylene and benzo[ghi]perylene which may contribute towards the development of an appropriate *in-situ* bioremediation techniques.

Methodology : Degradation of perylene (five ring) and benzo[ghi]perylene (six ring) was evaluated using yeast consortia under optimized conditions. Rate of biodegradation was tested with various kinetic models and intermediate metabolites were identified by GC-MS and FTIR analysis. Involvement of enzymes viz., 1,2-dioxygenase, 2,3-dioxygenase, catalase, laccase, lignin peroxidase and manganese peroxide were noted during PAHs degradation.

Results : The yeast consortium YC02 (*Hanseniaspora opuntiae* NS02, *Debaryomyces hansenii* NS03 and *Hanseniaspora valbyensis* NS04) degraded 67 % perylene at initial concentration 50 mg l⁻¹, whereas YC04 (*Rhodotorula* sp. NS01, *Debaryomyces hansenii* NS03 and *Hanseniaspora valbyensis* NS04) degraded 60 % benzo[ghi]perylene at initial concentration 40 mg l⁻¹ after 6 days. The consortium YC02 and YC04 exhibited best reaction by first order kinetics with half life period of 3.397 days and 4.218 days for degradation of perylene and benzo[ghi]perylene, respectively.

Interpretation : Based on the metabolites identified by GC-MS and FTIR analysis, possible pathways of perylene and benzo[ghi]perylene degradation were proposed. The results of present study revealed that application of yeast consortium can be a valuable approach towards remediation of high molecular weight PAHs having more benzene rings.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are potentially mutagenic and carcinogenic substances found in soil, surface water and sediments. They are chemically very stable and cannot be degraded easily. PAHs contain one or more benzene rings, the higher the benzene rings, the molecule is more persistent (de Boer and Wagelmans, 2016). Generally, the heavier (4-, 5-, and 6-ring) PAHs are more persistent than the lighter (2- and 3-ring) PAHs and have greater carcinogenic and other chronic impact potential (Hesham *et al.*, 2006).

Perylene is a high molecular weight polycyclic aromatic hydrocarbon (HMW PAH) compound having five benzene rings and reported as procarcinogen formed during the process of incomplete combustion of organic materials. It is phototoxic PAH (Irwin *et al.*, 1997) and found in marine sediments (Slater *et al.*, 2013); freshwater and river sediments (Fan *et al.*, 2011) and terrestrial sediments (Itoh and Hanari, 2010; Marynowski *et al.*, 2013). Benzo[ghi]perylene, another high molecular weight polycyclic aromatic hydrocarbon (HMW-PAH) compound having 6 benzene rings, is extremely recalcitrant to degradation (Zapfen *et al.*, 2009; Mohan *et al.*, 2011) and known for carcinogenic, mutagenic and teratogenic properties (Luch, 2005).

Several physico-chemical techniques such as incineration, thermal desorption, soil washing etc., are used for reclamation of PAHs polluted soil. But these methods are not of choice because of high cost and legislation constraints. Compared to conventional techniques, microbial technology seems to be more efficient, cost effective and eco-friendly for remediation of PAHs contaminated area (Li *et al.*, 2014). More reports are available on degradation of low molecular weight (LMW) PAHs compared to (HMW) PAHs. Though there are reports on degradation of perylene and benzo[ghi]perylene using fungi and yeast (Hesham *et al.*, 2006; Silva *et al.*, 2009; Winquist *et al.*, 2014; Garcia-Delgado *et al.*, 2015), detailed information on the mechanism of degradation, role of enzymes, degradation pathways are lacking. Therefore, the present work aimed to demonstrate the ability of yeast consortium elucidating the mechanism of degradation of perylene and benzo[ghi]perylene which may contribute towards the development of an appropriate *in-situ* bioremediation techniques.

Materials and Methods

Yeast consortium : In a previous study, four yeast strains were reported which were isolated from PAHs contaminated sites, Vellore, Tamil Nadu and identified as *Rhodotorula* sp. NS01, *Hanseniaspora opuntiae* NS02, *Debaryomyces hansenii* NS03 and *Hanseniaspora valbyensis* NS04 using molecular techniques (Mandal *et al.*, 2016). In the present study, each yeast consortium was formed by combining three identified yeast strains. Yeast consortia viz. YC01 (*Rhodotorula* sp. NS01, *Hanseniaspora opuntiae* NS02, *Debaryomyces hansenii* NS03); YC02

(*Hanseniaspora opuntiae* NS02, *Debaryomyces hansenii* NS03, *Hanseniaspora valbyensis* NS04); YC03 (*Rhodotorula* sp. NS01, *Hanseniaspora opuntiae* NS02, *Hanseniaspora valbyensis* NS04) and YC04 (*Rhodotorula* sp. NS01, *Debaryomyces hansenii* NS03, *Hanseniaspora valbyensis* NS04) were developed and used for the degradation study.

Screening of perylene and benzo[ghi]perylene degrading yeast consortium : Yeast inoculums were prepared in yeast extract peptone dextrose broth containing yeast extract; 10 g l⁻¹, peptone; 20 g l⁻¹, dextrose; 20 g l⁻¹ and agar; 20 g l⁻¹ with perylene (1.0 mg l⁻¹) and benzo[ghi]perylene (1.0 mg l⁻¹). For acclimatization study, the yeast consortium culture was transferred into fresh sterile medium with an increasing concentration of 1.0 to 10.0 mg l⁻¹, incubated at 28 ± 2 °C on the rotary shaker at 120 rpm for 5 days. To screen the potentiality of yeast consortium, yeast culture containing three yeast strains was suspended in mineral medium supplemented with perylene or benzo[ghi]perylene (10.0 mg l⁻¹) separately and incubated for 6 days. The cell suspensions were centrifuged at 8,400×g for 10 min at regular interval. The pellets were washed in methanol separately to remove residue of perylene and benzo[ghi]perylene. Then it was transferred into pre-weighed petri dishes and dried to constant weight at 105 °C for 45 min and dry weight of biomasses were calculated.

Optimization of growth parameters : To optimize the growth parameters during perylene or benzo[ghi]perylene degradation by yeast consortia, the flasks with 25 ml MM containing 10.0 mg l⁻¹ perylene and benzo[ghi]perylene were inoculated with 1 ml of YEPD broth culture of each yeast consortium (O.D.₆₀₀ = 0.1) and incubated at 30 ± 2 °C on the rotary shaker for 6 days. The effect of pH (5-9), temperature (10-40 °C), shaking speed (90-150 rpm), inoculum dosage (2-5 %), and initial perylene or benzo[ghi]perylene concentration (10-50 mg l⁻¹) during degradation were studied. During optimization of parameters, all others parameters were kept constant, except the optimizing parameter. Abiotic and biotic controls were maintained for each experiment. All the tests were conducted in triplicates.

Degradation of perylene and benzo[ghi]perylene : Experiments on perylene and benzo[ghi]perylene degradation were carried out in triplicates. Each flask was spiked with perylene (50 mg l⁻¹) and benzo[ghi]perylene (40 mg l⁻¹) containing yeast consortium at optimized dosage. The culture broths were incubated in an orbital shaker (130 rpm) for 6 days at 30 °C. The flasks were withdrawn at particular intervals for analysis of residual perylene and benzo[ghi]perylene and uninoculated culture flasks which were kept as control. The residual perylene and benzo[ghi]perylene in the culture medium was calculated.

Kinetics of perylene and benzo[ghi]perylene degradation : Degradation kinetics for the yeast consortia were performed in triplicates. The zero order (Wang *et al.*, 2002), first order (Agarrry *et al.*, 2013) and second order (Capellos and Bielski, 1972)

Fig. 1: (a) Growth curve of yeast consortium, YC02 in mineral broth and perylene biodegradation (%), abiotic control (O), cell dry weight (□) and residual perylene concentration (Δ). (b) Growth curve of yeast consortium, YC04 in mineral broth and benzo[ghi]perylene biodegradation (%), abiotic control (O), cell dry weight (□) and residual benzo[ghi]perylene concentration (Δ).

kinetics models were used to define the degradation of perylene and benzo[ghi]perylene in mineral medium.

Enzyme assays: Yeast cells grown in the presence of perylene and benzo[ghi]perylene were harvested at different time intervals (0, 2, 4 and 6 d) and were grown in mineral medium with 50 mg l⁻¹ perylene and 40 mg l⁻¹ benzo[ghi]perylene as carbon and energy source. The cultures were centrifuged (10,000 rpm, 15 min) at 25 °C and rapidly washed 3 times with 50 mM phosphate buffer (pH 7.0). Cells were disintegrated with a probe-type sonic oscillator for 10 min. The extract was centrifuged at 10,000 rpm at 4 °C for 30 min to remove whole cells and large debris and the collected supernatant was used immediately for the enzyme assays. Various enzymes viz., 1,2-dioxygenase (Dorn and Knackmuss, 1978), 2,3-dioxygenase (Bayly *et al.*, 1966), catalase (Flores *et al.*, 2014), laccase (Arora and Sindhu, 1985), lignin peroxidase (Li *et al.*, 2009) and manganese peroxidase (MnP) (Annudurai *et al.*, 2000) activities were assayed following the standard protocols collecting the samples at regular intervals of 0, 2, 4 and 6 days.

Characterization of degraded products by GC-MS and FT-IR analysis : Residual perylene or benzo[ghi]perylene and degraded products were determined by GC-MS chromatography and FTIR analysis. At regular intervals of 0, 2, 4 and 6 days of incubation, flasks were withdrawn for the analysis of degraded products. The residual perylene or benzo[ghi]perylene and also degraded products were extracted and analysed using GC-MS following the method of Arulazhagan *et al.* (2010). Standards from Sigma Aldrich and Supleco were used for perylene and benzo[ghi]perylene, respectively. GC-MS library search was used to confirm the metabolites without standards as available in the National Institute of Standard Technology (NIST) library, USA.

The FTIR spectra of perylene or benzo[ghi]perylene and degraded products were used to determine the vibrational frequency changes in the functional groups. IR spectroscopy was investigated with an IR affinity-1 FT-IR spectrophotometer (Shimadzu) using KBr pellets. The range of scanning was kept

between 4,000 to 500 cm⁻¹ and the spectrum resolution was 4 cm⁻¹.

Results and Discussion

Yeast consortia were screened for perylene and benzo[ghi]perylene degradation as sole carbon and energy source (Fig. 1). Based on the screening results, it was found that the yeast consortium, YC02 showed maximum perylene degradation (55 %), followed by YC01 (50 %), YC03 (48 %) and YC04 (45 %). In case of benzo[ghi]perylene, YC04 was found to show maximum degradation (46 %), followed by YC01 (40 %), YC02 (33 %) and YC03 (38 %). These results are consistent with the results obtained by others researches, who found that some fungal and bacterial strains preferred particular type of PAH as sole carbon source (Arulazhagan *et al.*, 2014; Balaji *et al.*, 2014; Alrumman *et al.*, 2016; Zafra *et al.*, 2017).

Yeast consortium, YC02 showed maximum degradation efficiency and cell biomass at pH 7, temperature at 30 °C, shaking speed at 130 rpm, inoculum dosage of 4 % and initial perylene concentration of 50 mg l⁻¹. Yeast consortium, YC04 showed maximum degradation efficiency and cell biomass at pH 7, temperature at 30 °C, shaking speed at 130 rpm, inoculum dosage of 5 % and initial benzo[ghi]perylene concentration of 40 mg l⁻¹ (Fig. 2 a, b). The yeast consortia were able to remove 50 mg l⁻¹ initial concentration of perylene in the following order: YC02 (67.0 %) > YC01 (62.0 %) > YC03 (59.0 %) > YC04 (56.0 %) and 40 mg l⁻¹ initial concentration of benzo[ghi]perylene in the order: YC04 (60.0 %) > YC01 (55.0 %) > YC02 (53.0 %) > YC03 (48.0 %), after 6 days of incubation period. Hesham *et al.* (2006) reported 70 % degradation of perylene at concentration of 6.03 mg kg⁻¹ and 78% degradation of benzo[ghi]perylene at concentration of 2.17 mg kg⁻¹ in 42 day using yeast consortium (*Candida maltose*, *Pichia anomala*, *Sporidiobolus salmonicolor*, *Pichia guilliermondii* and *Rhodotorula dairenensis*). Therefore, yeast consortia used in the present study showed better performance in terms of concentration of perylene and benzo[ghi]perylene, as well as

Table 1 : Kinetics parameters for the degradation of perylene (50 mg l⁻¹) and benzo[ghi]perylene (40 mg l⁻¹) by yeast consortium YC02 and YC04

Kinetic model	Parameters	YC02 (Perylene)	YC04(Benzo[ghi]perylene)
Zero order	Regression Equation	$C_t = -6.125t + 49.875$	$C_t = -4.393t + 41.79$
$C_t - C_0 = Kt$	K (d ⁻¹)	6.125	4.393
$T_{1/2} = C_0 / 2K_0$	$T_{1/2}$	4.082	4.553
	R ²	0.969	0.968
First order	Regression Equation	$\ln C_t = -0.204t + 3.980$	$\ln C_t = -0.164t + 3.7711$
$\ln C_t = K_1 t + \ln C_0$	K (d ⁻¹)	0.204	0.164
$T_{1/2} = \ln 2 / K_1$	$T_{1/2}$	3.397	4.218
	R ²	0.974	0.972
Second order	Regression Equation	$1/C_t = 0.0075t + 0.0152$	$1/C_t = 0.0065t + 0.0203$
$1/C_t = 1/C_0 + K_2 t$	K (d ⁻¹)	0.0075	0.0065
$T_{1/2} = 1/C_0 K_2$	$T_{1/2}$	92.4	106.615
	R ²	0.937	0.954

R² = Regression coefficient; K = Degradation rate constant; $T_{1/2}$ = Half life period

number of days needed for biodegradation. Based on the optimization study, the yeast consortium, YC02 for perylene and YC04 for benzo[ghi]perylene were selected for the further degradation study.

The kinetic data on degradation of perylene (50 mg l⁻¹) and benzo[ghi]perylene (40 mg l⁻¹) were fitted with three mathematical kinetic models (Fig.3a, b). The results showed that the degradation kinetics of perylene and benzo[ghi]perylene by yeast consortium, YC02 and YC04 can be described well by first order reaction kinetics with respect to the aqueous concentration of perylene and benzo[ghi]perylene. The highest regression coefficient (R²) values of 0.974 and 0.972 in first order kinetic model indicated that the degradation kinetics of perylene and benzo[ghi]perylene by yeast consortium, YC02 and YC04 follows first order reactions (Table 1). This implied that the removal of perylene and benzo[ghi]perylene by yeast consortia were time dependant process and degradation rate was directly proportional to substrate concentration (Deng *et al.*, 2012; Jin *et al.*, 2017). The calculated degradation rate constant K was 0.204 d⁻¹ and the theoretical half-life of perylene was 3.397 days, whereas calculated degradation rate constant K was 0.164 d⁻¹ and the theoretical half-life of benzo[ghi]perylene was 4.218 days. It was noted that higher the biodegradation rate constant, faster is the rate of biodegradation, and consequently the half-life times are lower. The calculated half-life of perylene and benzo[ghi]perylene, in the present study, was quite less.

On the initial phase of perylene as well as benzo[ghi]perylene breakdown, 1,2-dioxygenase showed profound activity both in the supernatant and cell lysate, whereas the reverse trend was observed in other degradative enzymes studied. Catalase, lignin peroxidase (LiP) and manganese peroxide (MnP) activity was found to be less on 2nd day, but was increased on 4th day and further decreased over incubation time. The activity of all the enzymes viz., catalase, laccase, lignin

peroxidase, manganese peroxide, 1,2-dioxygenase and 2,3-dioxygenase were more in cell lysate than in the cell supernatant, which implicated the intracellular nature of these enzymes (Fig. 4a, b). Dioxygenase helps in ring-cleavage as well as decyclize the aromatic compounds which allows the products to be channelled in the metabolic pathways of cells (Pérez-Pantoja *et al.*, 2010). Initial ring-cleavage of other HMW PAH (benzo[a]pyrene) by lignolytic enzymes such as 1,2-dioxygenase and 2,3-dioxygenase in *P. ostreatus* PO-3 has been reported by Mishra and Singh (2014).

Six possible metabolites were identified using NIST library identification program (Fig. 5a). Perylene, which eluted with a retention time of 26.3 min., was gradually degraded. Sample withdrawn on the day 2, indicated the presence of two different new possible intermediate peaks that eluted at 25.4 and 19.8 min and identified as benzeneacetic acid, 2-tridecyl ester (BATDE) and 1,2,4-benzenetric carboxylic acid 1,2-dimethyl ester (1,2,4-BCADME), both of which were detected on the day 4 as well, indicating that they were formed during the initial phase of degradation and were then transformed onto other compounds for further degradation. On 2nd day, the intensity of parent peak of perylene was reduced compared to parent compound. While another two apparent metabolites that were eluted at 21.4 and 15.2 min were seen on the 4th day of incubation and was confirmed to be hexanoic acid, 2-phenylethyl ester (HA,2-PEE) and butanoic acid, 2-methyl-2-phenyl ethyl ester (BA,2-MPEE), respectively, according to the NIST library. The intensity of perylene on 4th day was reduced compared to 2nd day. On 6th day, two more new degraded products, eluted at 17.3 and 12.5 min, were identified as n-propyl decyl ether (n-PDE) and propanoic acid, 2-methyl-2-ethyl-3-hydroxyhexyl ester (2-M-2-E-3-HHE), respectively. The parent compound intensity was reduced along with other newly degraded products which confirmed perylene degradation by the yeast consortium, YC02.

Fig. 2 : Optimization of growth parameters for biodegradation study in MM with (a) perylene and (b) benzo[ghi]perylene by different yeast consortia such as YC01(□), YC02(○), YC03(△), YC04(◇) and abiotic control (●). The effect of pH (5.0, 6.0, 7.0, 8.0 and 9.0), temperature (10 °C, 20 °C, 30 °C and 40 °C), shaking speed (90 rpm, 110 rpm, 130 rpm and 150 rpm), inoculums dosage with weight by volume percentage (2%, 3%, 4% and 5%) and initial perylene as well as benzo[ghi]perylene concentration (10 mg l⁻¹, 20 mg l⁻¹, 30 mg l⁻¹, 40 mg l⁻¹ and 50 mg l⁻¹) were evaluated for the period of 6 day

Fig. 3 : Kinetics models for biodegradation of (a) perylene by yeast consortium, YC02 and (b) benzo[ghi]perylene by yeast consortium, YC04 with zero order, first order and second order

Fig. 4 : Enzyme activities (U ml⁻¹) of (a) perylene degradation by yeast consortium, YC02 (b) benzo[ghi]perylene degradation by yeast consortium, YC04 in the culture supernatant and cell free extracts

Fig. 5 : (a) GC-MS analysis of perylene and its biodegraded metabolites by yeast consortium, YC02 at 2, 4 and 6 d. (b) Proposed perylene biodegradation pathway by yeast consortium, YC02. The dotted arrows represent transient products, which were not be detected by GC-MS analysis

Fig. 6 : (a) GC-MS analysis of benzo[ghi]perylene and its biodegraded metabolites by yeast consortium, YC04 at 2, 4 and 6 d. (b) Proposed benzo[ghi]perylene biodegradation pathway by yeast consortium, YC04. The dotted arrows represent transient products, which were not be detected by GC-MS analysis

Fig. 7 : FTIR analysis of (a) perylene before and after degradation by yeast consortium, YC02 and (b) benzo[ghi]perylene before and after degradation by yeast consortium, YC04 after 6 days

Based on the identified degraded products, a possible degradation pathway of perylene was proposed (Fig. 5b). The parent compound, perylene ($m/z = 252.3$) was initiated and gave rise to BATDE ($m/z = 207.3$) and 1,2,4-BCADME ($m/z = 191.1$). BATDE was further degraded giving rise to HA,2-PEE ($m/z = 128.2$), whereas 1,2,4-BCADME gave rise to BA,2-MPEE ($m/z = 104.2$) in which both compounds reduced the carbon chain length. The degradation pathway proceeded further, while HA,2-

PEE gave rise to a transient metabolite, n-PDE ($m/z = 44.1$) and BA,2-MPEE gave rise to 2-M-2-E-3-HHE ($m/z = 43$).

Benzo[ghi]perylene, which eluted with a retention time of 30.7 min, was gradually degraded into infinitesimal quantities (Fig. 6a). Sample withdrawn on day 2 indicated the presence of two different new possible intermediate peaks at 27.1 and 25.7 min and identified as megestrol acetate (ME) and 7-hydroxy-7,8,9,10-tetramethyl-7,8-dihydrocyclohepta[D,E]naphthalen(7-H-7,8,9,10-TM-7,8-DCHDEN), both of which were detected on day 4 as well indicating that they were formed during the initial phase of degradation and were then transformed into other compounds for further degradation. On 2nd day, the intensity of parent peak of benzo[ghi]perylene was reduced. While another three apparent metabolites that were eluted at 18.8, 23.8 and 17.9 min were seen on the 4th day of incubation and was confirmed to be 4-hexadecyclobenzoic acid (4-HCBA), 1,1-diphenyl-2-propanol (1,1-DP-2-P) and 4,6-di-tert-butylrescorinol (4,6-D-T-BR), respectively, according to the NIST library. The intensity of benzo[ghi]perylene on 4th day was reduced compared to 2nd day. On the 6th day, a new degraded product eluted at 14.1 min was identified as oxalic acid, 6-ethyloct-3-yl isohexyl ester (OA,6-EO-3YIE). The parent compound intensity was reduced compared to 4th day along with other newly degraded products which confirmed that benzo[ghi]perylene was degraded by the yeast consortium, YC04.

The degradation of parent compound, benzo[ghi]perylene ($m/z = 276.2$) was initiated to form, ME ($m/z = 207.2$) and 7-H-7,8,9,10-TM-7,8-DCHDEN ($m/z = 177.2$). Further by multi step reduction, 4-HCBA ($m/z = 138.1$) was formed which was reduced to OA,6-EO-3YIE ($m/z = 57.9$). The degradation pathway proceeded further, while 7-H-7,8,9,10-TM-7,8-DCHDEN gave rise to transient metabolites, 1,1-DP-2-P ($m/z = 167.2$) and 4,6-D-T-BR ($m/z = 73.1$) in which carbon rings were reduced (Fig. 6b). Meng *et al.* (2014) reported that degradation of PAHs may be enhanced with appropriate concentration of the metabolites.

FTIR spectra of control perylene (Fig.7a) showed the characteristic absorption peaks at 3047 cm^{-1} (=CH stretch in aromatic rings), $2850.79\text{--}2953.02\text{ cm}^{-1}$ (C-H stretching in cyclic ring), $1741.72\text{--}1921.1\text{ cm}^{-1}$ (weak overtone and combination bands in aromatic compounds), $1490.97\text{--}1587.42$ (variable aromatic ring stretching), $759.75\text{--}885.33\text{ cm}^{-1}$ (strong out of plane CH deformations in aromatic compounds) and $462.92\text{--}540.07\text{ cm}^{-1}$ (ring deformations in aromatic compounds). The second spectra illustrated, degraded perylene products by yeast consortium, YC02 showed a presence of 2985.81 cm^{-1} showed the strong CH stretch in methyl groups. Sharp absorption peak of 1735.93 cm^{-1} (very strong C=O stretch in esters groups), 1373.32 cm^{-1} (medium CH_3 deformations in isopropyl groups), 1234.44 cm^{-1} (varies from strong to very strong C-O-C stretch in ethers and also in esters groups), 1043.49 cm^{-1} (strong C-O stretch in CH-OH in cyclic alcohols) and 607.58 cm^{-1} (to strong O-C=O bending in carboxylic acids).

FTIR spectra of control benzo[ghi]perylene (Fig.7b) showed distinctive absorption peaks at 3020.25 cm^{-1} (C-H stretch in aromatic rings), 1730.15 cm^{-1} (weak overtone and combination bands in aromatic compounds), 752.24 cm^{-1} (strong out of plane CH deformations in aromatic compounds), 667.37 (strong CH out of plane deformations in cis disubstituted alkenes) and 447.35 cm^{-1} (medium-strong ring deformations in aromatic compounds). The second spectra illustrated, degraded benzo[ghi]perylene products by yeast consortium, YC04 showed a presence of 2880.11 cm^{-1} showed the presence of medium CH stretch in methyl groups in degraded products. Sharp absorption peak of 1756.17 cm^{-1} (strong C=O stretch in esters groups), 1465.73 cm^{-1} (medium CH_2 deformations in alkyl groups), 1281.46 cm^{-1} (strong C-O-C stretch in ethers and also in esters groups) and 1084.90 cm^{-1} (strong C-O stretch in CH-OH in cyclic alcohols and 640.70 cm^{-1} (strong O-C=O bending in carboxylic acids).

In the present study, out of four yeast consortia, two consortia (YC02 and YC04) were demonstrated as potential degrader of perylene and benzo[ghi]perylene in very less time period (6d). The potentiality of these two yeast consortia looks promising for the application in bioremediation technologies towards removal of high molecular weight polycyclic aromatic hydrocarbons (HMW PAHs) having five or six benzene rings.

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