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ROLE OF YEAST CONSORTIUM FOR THE REMEDIATION OF PERYLENE FROM AQUEOUS ENVIRONMENT: PROCESS OPTIMIZATION

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ABSTRACT

Perylene (PRL), a five- ring nuclear polycyclic aromatic hydrocarbon (PAH) has attracted attention because of its toxic properties. This study aimed to evaluate the efficiency of yeast consortium YC02 to remediate PRL in presence of ZnO nanoparticles and produced biosurfactant in the growth medium. Response surface methodology (RSM), 3- level five variables Box-Behnken design (BBD) was employed to optimize the factors viz. pH 7.0, temperature 30ºC, shaking speed 130 rpm, inoculum dosage 3% and zinc oxide nanoparticles (ZnO) concentration 2 g L^{-1} after a period of 6 days of incubation for the enhanced degradation of PRL (74 ± 0.01%) using yeast consortium. It was well in close agreement with the predicated value obtained by RSM model yield (74 ± 0.8%). Analysis of variance (ANOVA) showed F-value of 58.13, R^2 of 0.9790, probability of <0.0001 and coefficient of variation of 1.32 % confirmed the validity of the model. Degradation of PRL was assessed using GC-MS and FTIR analysis. Kinetic study demonstrated that PRL degradation fitted first order kinetic model. To the best of our knowledge, this is the first report on process optimization towards nanobioremediation of PRL using yeast consortium in presence of ZnO nanoparticles and produced biosurfactant in medium.

Keywords: Biosurfactant; nanobioremediation; PRL; polycyclic aromatic hydrocarbons (PAHs); response surface methodology (RSM); yeast consortium; ZnO nanoparticles

INTRODUCTION

Perylene (PRL) ($C_{10}H_{12}$), as a representative of polycyclic aromatic hydrocarbons (PAHs) has been reported as hazardous pollutant (**Donaldson *et al.*, 1953**). It is an extensively determined 5-ring PAH, derives from varied sediment environments such as marine sediments (**Slater *et al.*, 2013**), fresh water and river sediments and peats (**Hu *et al.*, 2014**). PRL has been identified in marine and terrestrial sediments ad well as in brown coals, crude oils and sedimentary rocks (**Marynowski *et al.*, 2015**). The occurrence of PRL is mainly associated with the terrestrial organic matter (**Stefanova *et al.*, 2013**). The terrestrial soil holds the precursors of PRL which are being transported by rivers to the coasts (**Varnosfaderany *et at*., 2014**). Although PRL has been the object of several environmental studies, report is very less on its toxicity. Respiratory tract lesions including tumors in respiratory tract have been reported as toxic effects of PRL (**Kephalopoulos *et al.*, 2014**). The carcinogenic effects of PRL in human and animals exposed orally or by inhalation have also been reported (**US EPA, 2007**). The coke oven workers were found to be affected by high concentration of PRL which reduced their levels of serum immunoglobins (**Irwin *et al.*, 1997**). **Cunha *et al.* (2006)** reported the toxicity of PRL on benthic bacteria and macro-fauna. The predominance of PRL over other PAHs has been found in marine sediments throughout the world at concentrations that are different from those of other PAHs (**Itoh and Hanari, 2010**).

There are many physico-chemical methods for remediation of PAHs which include chemical oxidation, photolysis, incineration, landfilling, volatilization and adsorption. But applications of these methods are limited due to certain drawbacks such as high operating cost, formation of toxic products etc. (**Gan *et al.*, 2009**). Microbial remediation is one of the most significant natural processes that can influence the fate of pollutants in both aquatic and terrestrial environment. Biodegradation is a promising method to remediate PAHs as it is inexpensive, environmental friendly and able to convert toxic substances into harmless products compared to conventional methods that need high cost and may produce hazardous by-products which can affect the environment (**Qin *et al.*, 2017**).

Biosurfactants have become one of the most versatile chemicals appropriate to be used for various industrial and environmental purposes. There are reports on bacteria and yeasts producing biosurfactants which are having lot of applications in various fields including remediation of number of pollutants(**Raza *et al.*, 2007**).

Recently, nanoparticles have been used as reductants or catalysts to improve various reactions due to their high surface areas and other characteristics (**Nezahat *et al.*, 2009**). In addition, effect of nanoparticles on microorganisms has additionally created great interests. Nanoparticles are able to assist microbial activities (**Shin and Cha, 2008**). But extremely limited studies have been conducted on bioremediation of pollutants in presence of nanoparticles and produced biosurfactants in the growth medium using microbes (**El-Sheshtawy and Ahmed, 2017**).

Remediation of PRL through biological method is receiving attention now-a-days. There are relatively few publications on successful microbial remediation of PRL (**Hesham *et al.*, 2006; Silva *et al.*, 2009; Mandal and Das, 2018**) . In addition, the use of microbial consortia is considered to be more stable and effective than using single organism because of their diversity and synergistic effect of metabolic activity that occur in microbial consortia (**Hesham *et al.*, 2006; Mishra *et al.*, 2014; Mandal and Das, 2018**). So far, no report is available on nanobioremediation of PRL in the presence of nanoparticles and produced biosurfactant in the medium using microbial consortium.

In the present study, an attempt has been made to optimize the remediation process using essential variables that enhance the biodegradability of PRL using 3-level Box- Behnken design which can provide a mathematical model showing the influence of each variable and their interactions. Experiments were conducted to study the biodegradation of PRL using yeast consortium in presence of ZnO nanoparticles and produced biosurfactant in the growth medium. To the best of our knowledge, this is the first study in which process optimization has been done towards nanobioremediation of PRL using yeast consortium in presence of ZnO nanoparticles and produced biosurfactant in medium.

MATERIALS AND METHODS

Microorganism

In our previous study, the yeast consortium YC02 was already reported as potential degrader of PRL (**Mandal and Das, 2018**) which consist of three yeast

isolates viz. *Hanseniaspora opuntiae* NS02, *Debaryomyces hansenii* NS03 and *Hanseniaspora valbyensis* NS04. So, YC02 was selected for the present study.

Synthesis and characterization of ZnO nanoparticles

The synthesis of ZnO nanoparticles was done following the standard method (**Litt and Almquist, 2009**). The ZnO nanoparticles were prepared by the precipitation technique using zinc chloride [$ZnCl_2.9H_2O$] (Sigma-Aldrich). In practical, 2.6 g of $ZnCl_2$ was dissolved in 1000 ml distilled water and precipitated using 33% dilute aqueous ammonium hydroxide solution (Sigma-Aldrich) at room temperature until the pH reached a value of 13. The slurry was then agitated for 30 min. The resultant precipitates were filtered and washed carefully, and the sample was dried at 100ºC for 12 h. The characterization of ZnO nanoparticles was done using UV spectroscopy, X ray diffraction (XRD), Fourier transform Infrared spectroscopy (FTIR) analysis, TEM and EDX analysis.

Production of biosurfactant in culture media

For biosurfactant production, a mineral salt medium (MSM) was prepared. Trace element solution containing (g L^{-1}): 0.116 of $FeSO_4.7H_2O$, 0.232 of H_3BO_3, 0.41 of $CoCl_2.6H_2O$, 0.008 of $CuSO_4.5H_2O$, 0.008 of $MnSO_4.H_2O$, 0.02 of $(NH_4)_6Mo_7O_{24}$ and 0.174 of $ZnSO_4$ were added to MSM (**Haghighat *et al*., 2008**). The yeast consortium YC02 was inoculated in 500 mL Erlenmeyer flasks containing 150 mL MSM of initial pH 7.0 and incubated at 30ºC for 3 days under shaking condition of 130 rpm.
Biosurfactant production by YC02 in MSM was confirmed by various tests viz. drop collapsing test (Bodour and Miller-Maier, 1998), methylene blue agar plate method (**Satpute *et al*., 2008**) and emulsification test (E_{24}) (**Bodour *et al.,* 2004**) following the standard procedures.

Biodegradation of PRL in presence of ZnO nanoparticles and produced biosurfactant

The biodegradation experiments were conducted in sterilized 500 mL Erlenmeyer flask containing 100 mL of sterilized mineral salt medium supplemented with PRL (50 mg L^{-1}) under different set of conditions as follows : (i) PRL + YC02 (ii) PRL + YC02+ produced biosurfactant (iii) PRL + YC02+ ZnO nanoparticles (0.5 g L^{-1}) (iv) PRL +YC02 + produced biosurfactant + ZnO nanoparticles (0.5 g L^{-1}). Flasks without inoculation were maintained as control. The residual PRL was extracted from the different set of conditions and biodegradation percentage was calculated.

Instrumental analysis

GC-MS analysis was done to determine the residual PRL and the degraded products in the culture broths (**Arulazhagan *et al*., 2010**). Flasks from different set of conditions were withdrawn after 6 days of incubation. The degraded products were extracted using ethyl acetate. The solvent was removed under vacuum by rotary evaporation (Superfit™ Rotary vacuum Digital bath) prior to analysis. Aliquots of 2-5 µL were injected directly for GC–MS analysis, (JEOL GC MATEII) using silica as stationary phase. The inlet temperature was 220ºC; oven temperature was increased from 50 to 250ºC at 10ºC rev min^{-1}; the GC interface temperature was 250ºC; carrier gas was nitrogen at a flow rate of 1.0 mL rev min^{-1}. Mass spectrum conditions had the ionization energy 70 eV, ion chamber temperature was maintained at 250ºC with tungsten filament which was used for the ionization of molecules. The concentration of PRL was calculated by comparing the peak areas of each treated sample with that of the peak area of the abiotic control. For identification of the degraded products, the mass spectra of the products formed were compared with respective mass spectra of authentic compounds and also with the mass profile of the same compound available in the National Institute of Standard Technology (NIST) library, USA.
The FTIR spectra of PRL and degraded products were used to determine the vibrational frequency changes in functional groups. The extended degraded products dissolved in ethyl acetate, were mixed with KBr and made in the form of pellets (13 mm in diameter and 1 mm thickness). IR spectroscopy was investigated with the IR affinity-1 FT-IR spectrophotometer (Shimadzu). The scanning wavenumber ranged from 4,000 to 400 cm^{-1} and the spectral resolution was 4 cm^{-1}.

Process optimization

Response surface methodology (RSM) using Box-Behnken design (BBD) was used for optimization of parameters and to determine the significant single factors, interactions and quadratic terms. Each factor was varied at three different levels -1, 0 and +1 signifying low, medium and high values. The minimum and maximum ranges of pH, temperature, shaking speed, inoculum dosage and zinc oxide nanoparticle concentrations were determined as shown in Table 1. A design of 46 experiments were formulated and the experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of production medium (PRL 50 mg L^{-1}). The experiments were performed twice to optimize the levels of the selected variables viz., pH (A), temperature (B), shaking speed (C), inoculum dosage (D) and zinc oxide nanoparticle concentrations (E). The range of the variables was chosen based on preliminary experiments. The 3D contour plots were prepared to evaluate the optimized parameters, which influence the response. The respective responses were analyzed by using a second order polynomial equation, and the data were fitted to the equation by multiple regression procedures. Later, an experiment was conducted in triplicates using the optimum values for variables given by response surface optimization to validate the predicted value and the observed value of the responses. The results of the experimental design were analyzed and interpreted using Design-Expert version 11.0 (Stat-Ease Inc. Minneapolis, MN, USA) statistical software (**Sahoo and Gupta, 2012**).

Table 1 Independent factors and its level used in response surface design for PRL biodegradation

Factors	Name	Level (0)	Low level(-1)	High level(+1)
A	pH	7	5	9
B	Temperature (°C)	30	10	50
C	Shaking speed (rpm)	130	110	150
D	Inoculum dosages (%)	3	1	5
E	ZnO nanoparticle concentration (g L^{-1})	2	1	3

Kinetic studies

Degradation kinetics was performed in triplicates. The zero order (**Wang *et al*., 2002**), first order (**Agarry *et al*., 2013**) and second order (**Capellos and Bielski, 1972**) kinetic models were used to define the degradation of PRL in mineral medium.

RESULTS AND DISCUSSION

Characterization of ZnO nanoparticles

UV-Visible Spectroscopy analysis of ZnO nanoparticles

The optical characterization of the sample was recorded on UV-Vis absorption spectrophotometer showed in Figure 1a. The UV-Visible absorption spectroscopy of ZnO nanoparticles in deionized water exhibited maximum absorption at two different wavelengths of 240 nm and 390 nm which clearly indicated the gradual formation of ZnO nanoparticles. Similar result was reported by **Kulkarni and Shirsat (2015).**

XRD analysis of ZnO nanoparticles

The XRD pattern of ZnO nanoparticle exhibited well-defined peaks at 2θ values of 13.13, 15.28, 26.13, 26.90, 33.31, 38.94 and 59.69 which correspond to the 010, 011, 113,104, 213, 123 and 401 planes respectively (Figure 1b). The intensity of ZnO nanoparticle peaks at 2θ values of 15.28, 26.90, 33.31 and 59.69 reflected high degree of crystallinity in nanoparticles. Similar result was demonstrated by **Kumar and Rani (2013).**

FTIR analysis of ZnO nanoparticles

The FTIR spectrum of dispersed ZnO nanoparticles in deionized water was shown in Figure 1c. Infrared studies were carried out in order to ascertain the purity and nature of the zinc oxide nanoparticles. Metal oxides generally give absorption bands in fingerprint region i.e. below 1000 cm^{-1} arising from inter-atomic vibrations. The peaks at observed at 3257.77, 3165.19, 3101.54 cm^{-1} were due to O-H stretching vibration arising from hydroxyl groups from the water on nanoparticles. The absorption peaks at 1548.84, 1498.69, 1361.03, 1354.03, 1039.63 and 947.05 cm^{-1} were due to de-ionized water used as solvent. The absorption peaks at 690.52, 514.99, 480.29 cm^{-1} were corresponding to the Zn-O bond stretching and deformation vibration, respectively. Similar FTIR spectra were observed in case of zinc oxide nanoparticles (**Parthasarathi and Thilagavathi, 2011; Kumar and Rani, 2013**).

TEM and EDX analysis of ZnO nanoparticles

The morphological and structural properties of the prepared nanoparticle are illustrated in the TEM images (Figure 1d). The ZnO nanoparticle particles are highly agglomerated with roughly the average particle size of 45 mm. The elemental analysis of ZnO nanoparticle was performed using energy dispersive

X-ray (EDX) analysis (Figure 1e). The EDX spectrum of ZnO nanoparticle indicated the presence of Zn and O as present on ZnO nanoparticle. The peak of Zn and O were noted on EDX which confirmed ZnO nanoparticle.

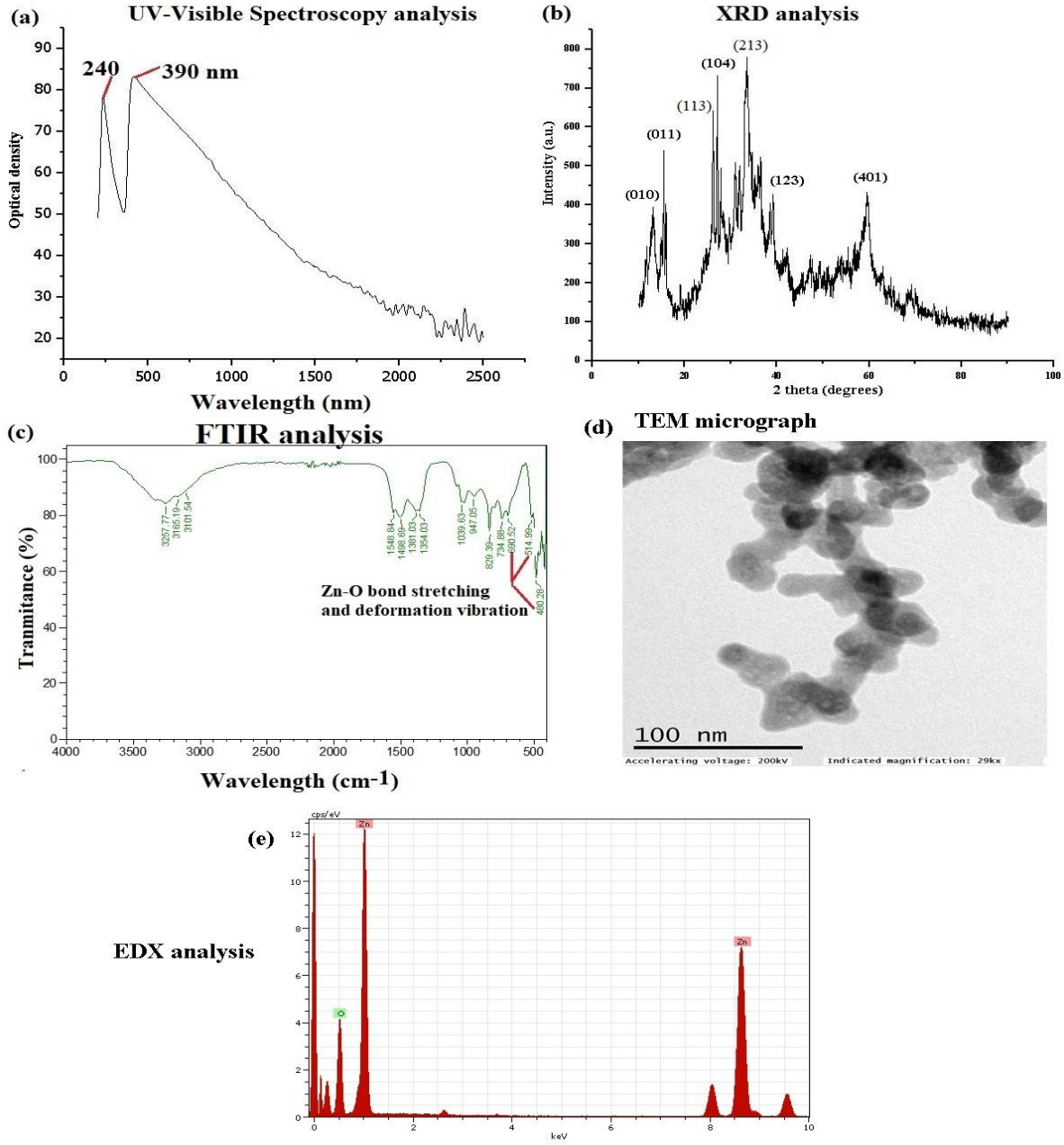

Figure 1 Characterization of ZnO nanoparticle: (a)UV-Visible Spectroscopy analysis of ZnO nanoparticle, (b) XRD analysis of synthesized ZnO nanoparticles, (c) FTIR analysis of synthesized ZnO nanoparticle, (d) TEM micrographs of synthesized ZnO nanoparticle and (e) EDX of synthesized ZnO nanoparticle.

Biosurfactant production

Biosurfactant produced by yeast consortium (YC02) in the MS medium was tested by methylene blue agar test, drop collapsing test and emulsification index (%). The positive results of all the tests confirmed the biosurfactant producing ability of YC02. The flat drop appearance in micro titer plate confirmed the positive results for drop collapse test. Dark blue halo zone in the methylene blue agar plate supplemented with CTAB confirmed the presence of anionic biosurfactant (Figure not shown). The emulsification index was found to be 61.7%.

GC-MS analysis for PRL biodegradation

The degradation of PRL (50 mg L^{-1}) by yeast consortium YC02 in MSM was found to be 67.0% after 6 days of incubation. Improvement in degradation (68.3%) was noted when MSM was supplemented with ZnO nanoparticles (0.5 g L^{-1}). Maximum degradation of PRL (70.0%) was observed in presence of ZnO nanoparticles (0.5 g L^{-1}) and produced biosurfactant in MSM (Figure 2a). This implementation proved that ZnO nanoparticles were capable of assisting the microbial activities which agree with other studies (**Shin and Cha, 2008**). **Hesham *et al.* (2006)** reported the degradation of PRL (6.03 mg Kg^{-1}) by a mixture of five yeast strains which was found to be 70% after a period of 42 days. The soil fungi viz. *Aspergillus* sp. and *Achremonium* sp. were also reported to be capable of degrading PRL 37.0% and 20.5% respectively after a period of 30 days at the concentration 0.05 (w v^{-1}) (**Silva *et al.*, 2009**). Therefore, yeast consortium YC02 used in the present study was found to be more efficient in degrading PRL at much higher concentration (50 mg L^{-1}) within a short period (6 days) compared to the earlier reports. According to the Figure 2a, it can be concluded that the presence of ZnO nanoparticles could enhance the ability of yeast consortium YC02 in terms of improving the biosurfactant properties which followed by enhancing the biodegradation process. So far, extremely limited studies have been reported on combined effect of nanoparticles and biosurfactant towards degradation of environmental pollutants (**Zhang *et al.*, 2011**).

FT-IR analysis

FTIR spectra of control perylene (Figure 2b) showed the characteristic absorption peaks at 3047 cm^{-1} (=CH stretch in aromatic rings), 2850.79-2953.02 cm^{-1} (C-H stretching in cyclic ring), 1741.72-1921.1 cm^{-1} (weak overtone and combination bands in aromatic compounds), 1490.97-1587.42 cm^{-1} (variable aromatic ring stretching), 759.75-885.33 cm^{-1} (strong out of plane CH deformations in aromatic compounds) and 462.92-540.07 cm^{-1} (ring deformations in aromatic compounds). The second spectra illustrated, degraded perylene products by yeast consortium, YC02 showed a presence of 2970.38-2796.78 cm^{-1} representing H-bonded OH stretch in carboxylic acid. Sharp absorption peak of 1739.79 cm^{-1} (overtone and combination bonds in aromatic compounds), 1637.56 cm^{-1} (C=O stretching, enol

form in β-kentone esters), 1537.27 cm^{-1} (aromatic ring stretching), 1427.32 cm^{-1} (in plane OH bonding in carboxylic acids), 1369.46 cm^{-1} (medium CH_3 deformations in isopropyl groups), 1215.15 cm^{-1} (C-O-C antisym stretch in vinyl ethers), 1004.91 cm^{-1} (ring breathing mode of carbon ring in cyclic compounds), 954.76 cm^{-1} (=CH out of plane deformation in vinyl compounds), 615.29 cm^{-1} (C-OH out of plane deformations in alcohols) and 572.86-534.28 cm^{-1} (ring deformations in aromatic compounds). These results suggest that the parental compound has undergone significant changes after degradation.

Process optimization

A statistical tool, three level 5 variables Box-Behnken Design was implemented to enhance the biodegradation of PRL using yeast consortium (YC02). The factors were optimized by BBD with six central points, the response PLR biodegradation (%) was studied and the second-order polynomial equation was given below:

$Y= 74.2200 + 0.8750A - 0.25B - 0.125C + 0.3125D - 0.5625 E + 3.25AB + 3.00AC -1.50 AD + 3.25AE – 0.25BC + 1.25 BD + 0.75 BE + 0.25 CD - 2.5 CE + 1.25 DE - 5.19 A^2 - 5.52 B^2 -7.35 C^2 - 4.44D^2 - 3.44 E^2$

Where, Y was representing PRL biodegradation (%) as response and A, B, C, D and E were coded terms for the five test variables viz. pH, temperature, shaking speed, inoculum dosages and ZnO nanoparticle concentration respectively. The lack of fit analysis was found to be not significant which considered that the model is fit.

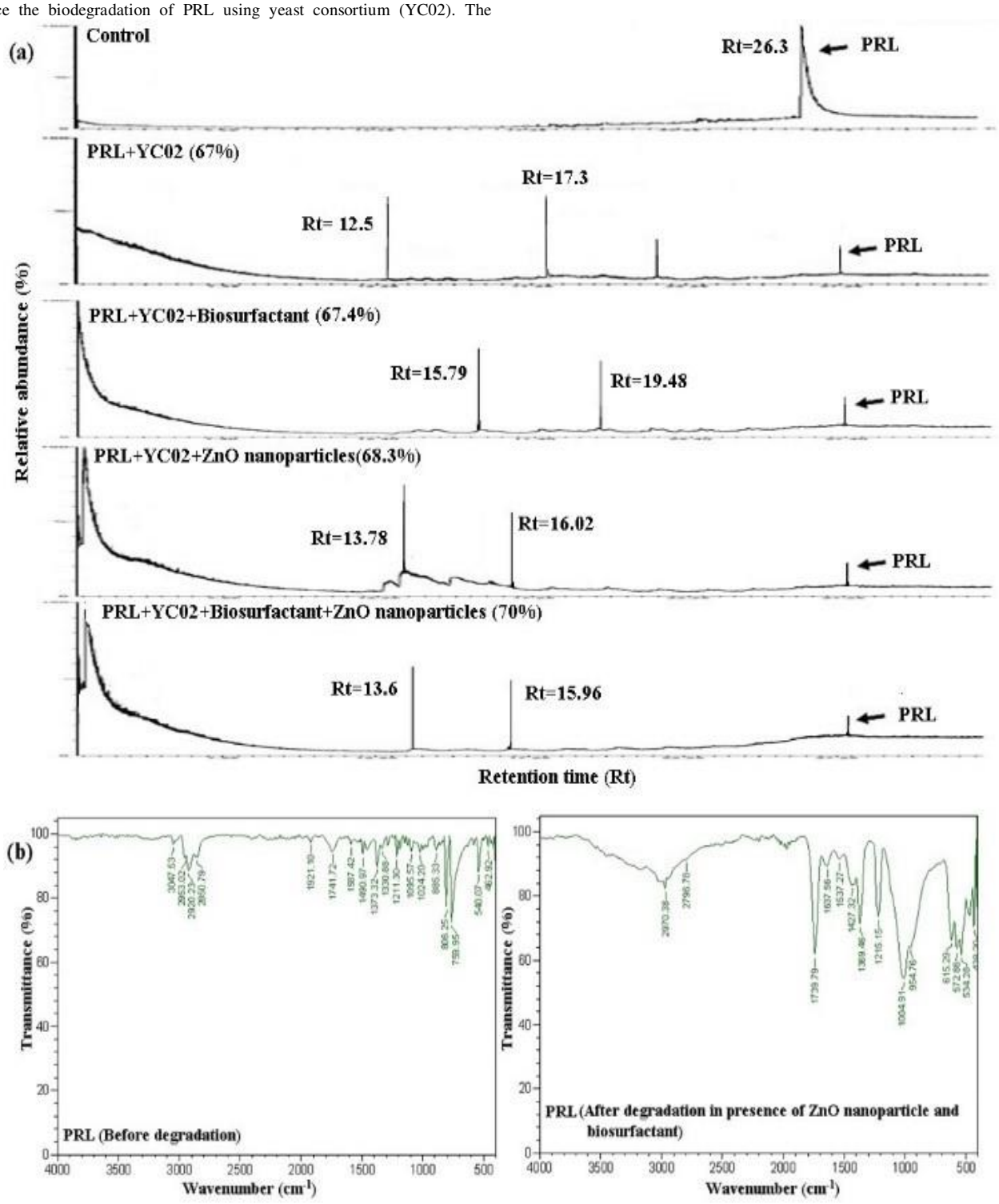

Figure 2 (a) GC-MS analysis of PRL degradation under different sets of condition after 6 days. (b) FT-IR spectrum of PRL before degradation and after degradation in presence of ZnO nanoparticle and biosurfactant.

The total determination coefficient R-Squared value was found to be 0.9790, indicating a realistic fit of the model to the experimental data (Table 2). This also indicates that 97% variation of response can be elucidated effectively and approves that 3% of the variations occur while performing the experiments. The ratio of 28.59 indicated an adequate signal, thus this model can be used to navigate the design space (Table 2). In this case, A, E, AB, AC, AD, AE, BD, CE, DE A^2, B^2, C^2, D^2 and E^2 were found to be significant model terms ($p< 0.05$) as tabulated in Table 2. When considered in linear terms, variables namely pH and ZnO nanoparticle concentration had shown the highest influence on PRL

biodegradation, as compared to temperature, shaking speed and inoculum dosages. When considered with respect to squared terms, all the variables had positive significance on PRL biodegradation as tabulated in Table 2. The interactive effect of variables, AB, AC, AD, AE, BD, CE and DE were found to be the most significant in response as shown in Figure 3. In Figure 3a, the response plot AB (pH vs temperature) indicated a significant reduction from acidic to alkaline pH and from lower (10°C) to higher temperature on PRL biodegradation. The maximum PRL biodegradation was observed at pH 7 and at temperature of 30°C. The response plots of AD (pH vs inoculum dosages) and BD (temperature vs inoculum dosages) were found to be significant and maximum degradation was observed at 3% inoculum as shown in Figure 3c, e. The response plots of AC (pH vs shaking speed) was found to be significant and maximum degradation was observed at 120 rpm as shown in Figure 3b.

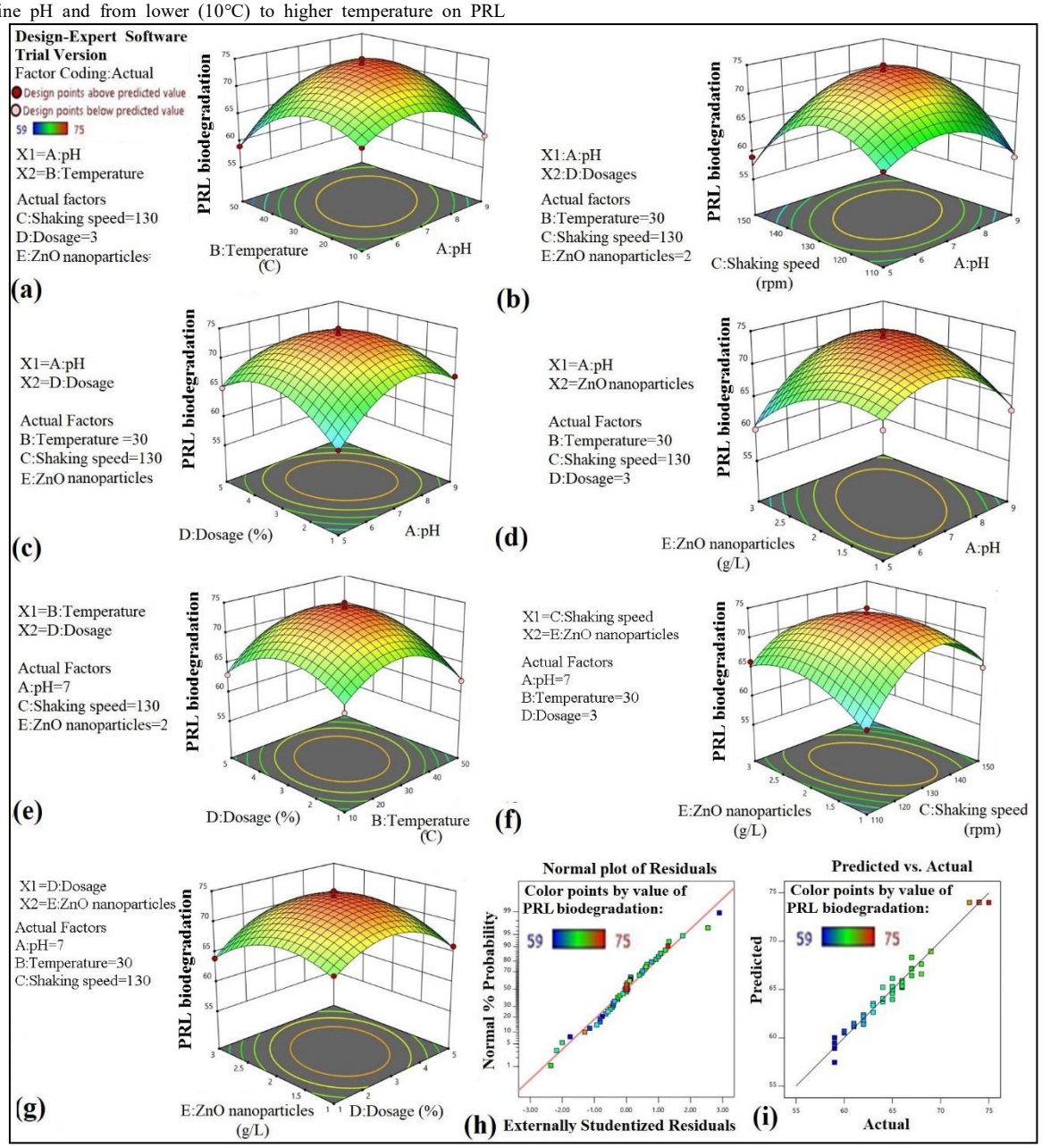

Figure 3 3-D interactions between the different variables for response (PRL biodegradation %) where, (a) pH vs. Temperature (AB) (b) pH vs. Shaking speed (AC) (c) pH vs. Dosage (AD) (d) pH vs. ZnO nanoparticles (AE) (e) Temperature vs. Dosage (BD) (f) Shaking speed vs. ZnO nanoparticles (CE) (g) Dosage vs. ZnO nanoparticles (DE) (h) Normal plot of residuals (i) Predicted vs. actual plot.

The response plot of AE (pH vs ZnO Nps), CE (shaking speed vs ZnO Nps) and DE (inoculum dosage vs ZnO Nps) were found to be significant and maximum PRL biodegradation was observed at 2.0 gL^{-1} ZnO nanoparticle as shown in Figure 3d, f, g. The supplementation of ZnO nanoparticle in the production medium had showed significant impact on PRL biodegradation (%) using the yeast consortium. However, a significant reduction in the PRL degradation was observed at higher concentration of ZnO nanoparticles (3 g L^{-1}). This could be because of the addition of high amount of ZnO Nps which become toxic to yeast cells. Similar trend by **El-Sheshtawy and Ahmed (2017)** was reported where crude oil was degraded using *Bacillus licheniformis* in the presence of different concentration nanoparticles and produced biosurfactant.

Table 2 ANOVA for Response Surface Quadratic Model (Response: PRL biodegradation %)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	852.64	20	42.63	58.13	< 0.0001	significant
A-Ph	12.25	1	12.25	16.70	0.0004	significant
B-Temperature	1.00	1	1.00	1.36	0.2539	
C-Shaking speed	0.25	1	0.25	0.34	0.5645	
D-Dosage	1.56	1	1.56	2.13	0.1568	
E-ZnO nanoparticle	5.06	1	5.06	6.90	0.0145	significant
AB	42.25	1	42.25	57.61	< 0.0001	significant
AC	36.00	1	36.00	49.09	< 0.0001	significant
AD	9.00	1	9.00	12.27	0.0018	significant
AE	42.25	1	42.25	57.61	< 0.0001	significant
BC	0.25	1	0.25	0.3409	0.5645	
BD	6.25	1	6.25	8.52	0.0073	significant
BE	2.25	1	2.25	3.07	0.0921	
CD	0.2500	1	0.2500	0.3409	0.5645	
CE	25.00	1	25.00	34.09	< 0.0001	significant
DE	6.25	1	6.25	8.52	0.0073	significant
A^2	234.85	1	234.85	320.25	< 0.0001	significant
B^2	266.00	1	266.00	362.73	< 0.0001	significant
C^2	472.00	1	472.00	643.64	< 0.0001	significant
D^2	171.85	1	171.85	234.34	< 0.0001	significant
E^2	103.12	1	103.12	140.62	< 0.0001	significant
Residual	18.33	25	0.7333			
Lack of Fit	16.33	20	0.8167	2.04	0.2201	not significant
Pure Error	2.00	5	0.4000			
Cor Total	870.98	45				
Std. Dev.	0.8563					
Mean	64.98					
C.V. %	1.32					
R^2	0.9790					
Adjusted R^2	0.9621					
Predicted R^2	0.9217					
Adeq Precision	28.5889					

A statistical model was validated by executing point prediction tool of BBD from an optimum value of all the 5 variables A, B, C, D and E. The actual PRL biodegradation (74.00 ± 0.01%) was in close agreement with the predicted value (74.00 ± 0.8%) indicating the validity of the model (Table 3). Maximum biodegradation of PRL was found to be 74.0 ± 0.01 (%) at central values of all the factors viz., pH (7.0), temperature (30ºC), shaking speed (130 rpm), ZnO nanoparticle (2 g L^{-1}) and inoculum dosages (3%). The normal plot for residuals and predicted vs actual plots were represented (Figure 3h-i) respectively. Thus, the biodegradation of PRL by YC02 was found to be increased from 70.0% to 74.0% in aqueous medium under optimized condition using BBD.

Table 3 Actual versus predicted value for Response: PRL biodegradation (%)

Std	Run	Factor 1 A:Ph	Factor 2 B:Temperature (degree)	Factor 3 C:Shaking speed (rpm)	Factor 4 D: Dosage (%)	Factor 5 E:ZnO nanoparticle (g L^{-1})	Response: PRL biodegradation (%) Actual value	Predicted values
42	1	0	0	0	0	0	74	74.00
6	2	0	0	1	-1	0	62	61.52
24	3	0	1	1	0	0	60	60.50
44	4	0	0	0	0	0	74	74.00
16	5	1	0	1	0	0	66	65.21
9	6	0	-1	0	0	-1	68	66.60
3	7	-1	1	0	0	0	59	58.92
29	8	0	0	-1	0	-1	62	61.40
36	9	1	0	0	0	1	69	68.94
20	10	0	0	0	-1	1	67	67.13
33	11	-1	0	0	0	-1	67	68.31
13	12	-1	0	-1	0	0	64	63.71
17	13	0	0	0	-1	-1	68	67.63
28	14	1	0	0	1	0	62	62.23
27	15	-1	0	0	1	0	65	65.31
39	16	0	-1	0	1	0	63	63.35
25	17	-1	0	0	-1	0	62	61.69
8	18	0	0	1	1	0	63	62.65
41	19	0	0	0	0	0	73	74.00
21	20	0	0	-1	0	0	61	61.25
45	21	0	0	0	0	0	75	74.00
46	22	0	0	0	0	0	74	74.00
15	23	-1	0	1	0	0	59	57.46
2	24	1	-1	0	0	0	61	61.17
12	25	0	1	0	0	1	65	64.98
35	26	-1	0	0	0	1	60	60.69
1	27	-1	-1	0	0	0	66	65.92
34	28	1	0	0	0	-1	63	63.56
43	29	0	0	0	0	0	74	74.00
31	30	0	0	-1	0	1	66	65.27
10	31	0	1	0	0	-1	65	64.60
30	32	0	0	1	0	-1	65	66.15
11	33	0	-1	0	0	1	65	63.98

40	34	0	1	0	1	0	66	65.35
4	35	1	1	0	0	0	67	67.17
14	36	1	0	-1	0	0	59	59.46
18	37	0	0	0	1	-1	66	65.75
22	38	0	1	-1	0	0	61	61.25
32	39	0	0	1	0	1	59	60.02
38	40	0	1	-1	-1	0	62	62.23
23	41	0	-1	1	0	0	61	61.50
5	42	0	0	-1	-1	0	62	62.27
19	43	0	0	0	-1	1	64	64.00
7	44	0	0	-1	1	0	62	62.40
26	45	1	0	0	-1	0	67	66.44
37	46	0	0	0	-1	0	64	65.23

Kinetic studies

The kinetic study was performed applying the predicted optimum conditions. The kinetic data on degradation of PRL (50 mg L^{-1}) was best fitted with the first order kinetic model in case of all set of conditions. The highest regression coefficient (R^2) values of (0.9938) of PRL degradation was noted in case of biosurfactant producing YC02 in presence of ZnO nanoparticles as shown in Table 4. The calculated degradation rate constant (K) of PRL is 0.206 d^{-1} and the theoretical half-life of PRL is 3.364 days implied that the removal of PRL by yeast consortium was time dependent process and degradation rate was directly proportional to substrate concentration (**Jin *et al.*, 2017**).

Table 4 Kinetic parameters for the degradation of perylene (PRL) using yeast consortium YC02

Set of conditions	Kinetics equation of degradation	Rate constants of degradation			
		R^2	K(d^{-1})	$T_{1/2}$ (days)	
	Zero order				
PRL + YC02	C_t = -5.520t + 48.91	0.9935	5.520	4.529	
PRL + YC02 + biosurfactant	C_t = -5.565t + 48.77	0.9918	5.565	4.492	
PRL + YC02 + ZnO nanoparticles (0.5 g L^{-1})	C_t = -5.885t + 48.43	0.9735	5.885	4.428	
PRL + YC02 + biosurfactant + ZnO nanoparticle (0.5 g L^{-1})	C_t = -5.960t + 48.38	0.9741	5.960	4.195	
	First order				
PRL + YC02	$\ln C_t$ = -0.1822t + 3.9433	0.9891	0.1822	3.804	
PRL + YC02 + biosurfactant	$\ln C_t$ = -0.1847t + 3.9401	0.9920	0.1847	3.752	
PRL + YC02 + ZnO nanoparticles (0.5 g L^{-1})	$\ln C_t$ = -0.2008t + 3.9303	0.9929	0.2008	3.451	
PRL + YC02 + biosurfactant + ZnO nanoparticle (0.5 g L^{-1})	$\ln C_t$ = -0.2060t + 3.9321	0.9938	0.2060	3.364	
Second order					
PRL + YC02	$1/C_t$ = 0.0066t + 0.0166	0.9316	0.0066	3.030	
PRL + YC02 + biosurfactant	$1/C_t$ = 0.0067t + 0.0167	0.9379	0.0067	2.985	
PRL + YC02 + ZnO nanoparticles (0.5 g L^{-1})	$1/C_t$ = 0.0076t + 0.0168	0.9720	0.0076	2.632	
PRL + YC02 + biosurfactant (0.5 g L^{-1})	$1/C_t$ = 0.0079t + 0.0166	0.9697	0.0079	2.532	+ ZnO nanoparticles

CONCLUSION

To conclude, anionic biosurfactant was produced by yeast consortium YC02 which could enhance the degradation of perylene (PRL) in the growth medium. However, the best improvement on PRL degradation evaluated to 70.0% which was recorded in the presence of biosurfactant and ZnO nanoparticles both. In addition, statistical optimization of growth parameters remarkably enhanced the PRL degradation (74 %) by YC02 in presence of ZnO nanoparticles and produced biosurfactant which establishes the novelty of our work. Results suggested the potential applicability of the yeast consortium YC02 for the bioremediation of PRL contaminated sites using biosurfactant and specific concentration of ZnO nanoparticles. Kinetic studies suggested that degradation of PRL by yeast consortium was time dependent process and degradation rate was directly proportional to substrate concentration. According to the best of our knowledge, this is the first report on nanobioremediation of PRL, a high molecular weight PAH using yeast consortium. Further work on application of YC02 in nanoremediation of PRL from the real-world contaminated environment is underway in order to ascertain its relevance in pollution control.

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