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Simultaneous *o*-cresol degradation and biosurfactant production by indigenous bacterial monoculture: kinetics and genotoxic risk assessment

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ABSTRACT

Use of a novel bacterial isolate in environmental cleanup from toxic *o*-cresol along with valuable biosurfactant generation has been simultaneously explored, and a relevant correlation between the two has been obtained in this study. Paper pulp effluent from Durgapur, West Bengal, India, was screened to obtain indigenous *o*-cresol-tolerating bacteria, identified to be *Pseudomonas fluorescens* (NITDPY Accession no. KM111571). The bacteria showed significant stimulatory growth in *o*-cresol concentration of 5 mg L^{-1} and tolerance up to 200 mg L^{-1}. Monod model fitted well at initial lower concentrations, whereas at higher concentration range (100–1,000 mg L^{-1}), model Aiba was found to be the best fit. The rate of degradation of *o*-cresol showed observable correlation with bacterial growth fitting best with Aiba model. The role of enzymes in *o*-cresol-degrading pathway was studied, where enzyme catechol 1,2-dioxygenase was found to be involved in *ortho*-cleavage pathway. The role of interesting amphiphillic microbial metabolites and biosurfactants participating in *o*-cresol degradation was explored. Biosurfactant was being produced by the bacterial isolate and was revealed by specific assays (emulsification assay, oil spreading assay, foam height assay, etc.). Surface tension of water was lowered down to 44 mN m^{-1}. Thin layer chromatography and Fourier transform infra-red spectroscopy studies confirmed the specific characteristics coinciding with the nature of biosurfactants. Induction of biosurfactant production via *o*-cresol was found to be 2.5 fold. Nonhazardous eco-friendly nature of the biotreatment has been unwrapped by cytotoxicity and genotoxicity assays, where reduction of toxicity was prominently detected in bacteria-treated *o*-cresol on *Allium cepa* as compared with untreated control. Comet assay for genotoxicity assessment, which has been rarely explored in the area of study, revealed similar results.

Keywords: Pseudomonas fluorescens NITDPY; Growth kinetics; Degradation kinetics; *o-cresol* degradation pathway; Cytotoxicity; Genotoxicity; Biosurfactants

1. Introduction

Management of phenol and phenolic derivatives containing wastewater is a key challenge to industries in today's global scenario both in terms of economic and environmental prospects. Methylated derivatives of phenol, viz., *ortho*-, *meta*-, and *para*-cresol released from industrial effluents lead to effective groundwater pollution [1]. *O*-cresol, being the most toxic among the three isomers, is regrettably more frequently present in industrial effluents due to its wide usage in industries as a solvent and as intermediate for herbicides, dyes, resins, pharmaceuticals, cosmetics production, etc. [2]. The enhanced toxicity in comparison with phenol is

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due to the presence of methyl groups as per P_{ka} and P_{ow} values (octanol water partition coefficient [3]) and hence is listed as stable priority toxicants by United States Environmental Protection Agency. The permissive level of *o*-cresol in industrial effluent as set by the regulatory bodies is 5 mg L^{-1}, which is quite low due to its extreme levels of toxicity hence its removal is obligatory for restoring welfare of the ecosystem [4]. Besides being too expensive, the conventional treatment methods used by the industries fail to meet the set standards as well, thus effective and eco-friendly immediate alternative treatment methods like bioremediation are in huge demand.

Harnessing microbes that are potent *o*-cresol degraders has become an attractive option over traditional physicochemical treatments that were being used by the industries due to its cost-effective, energy-saving, and eco-friendly nature. A wide range of fungi and bacteria have been reported till date as degraders of hazardous pollutants like phenol and cresol [5]. Previous reports on indigenous *Pseudomonas putida* in aerobic reactors by Maeda et al. 2005 [6], specific *Pseudomonas putida* DSM 548 (pJP4) by Saravanan et al. 2008 [7], and *Pseudomonas aeruginosa* S8 by Gallego et al. [8] and reports by many others suggest that a broad cascade of bacteria of genus *Pseudomonas* to be present in waste-activated sludge are successful *o*-cresol degraders, although inquisition on the kinetics of *Pseudomonas*-mediated *o*-cresol degradation has not yet been done or reported so far in spite of being a crucial field of study for its application in industries as well as large-scale operations.

A large number of intermediates are generated during the course of phenolic derivative degradation, which plays significant roles as pioneers in enzymatic pathways thus further enhancing its degrading potentials. One such important intermediate is catechol [9], which induces a couple of enzymes, namely, catechol 1,2-dioxygenase belonging to the *ortho* ring fission pathway and catechol 2,3-dioxygenase belonging to the *meta* ring fission pathways [10]. The aerobic or anaerobic degradation of phenolic compounds by different bacterial strains of *Pseudomonas* genus following either *ortho*- or *meta*-cleavage pathway has been well documented [11,12].

However, the reduced bioavailability and solubility of the pollutants recognized as substrates are the reason for the diminished bio-removal potential by the microbes. Biosurfactants are wonder molecules that enhance the ability of the microbes to get acclimatized to pollutants, and hence, they play major roles in environmental cleanup. [13]. They are bioemulsifiers and are often exopolysaccarides of marine origin [14]. To enhance the bioavailability of the pollutants, surfactants may play a crucial role as studied in case azo dye decolorization phenol bisphenol and hydrocarbon degradation [15–17]. Reports state the induction of specific enzymes by biosurfactants during treatment of pollutants [18,19]. Rhamnolipids mitigate the negative effects of phenol on fungal growth and consequently improve laccase production and phenol degradation by Zhou [20]. Various reports on biosurfactants being produced by fungus like *Candida lipolytica* [21] have already been reported, but production of the same by various bacterial species is also a thriving area of research. Several reports have revealed *Pseudomonas* sp. to be efficient producers of biosurfactants [22,23], which explain the potential of these strains in the removal of toxic pollutants as well as their roles in oil recovery [24]. Besides, biosurfactant-producing bacteria growing on cheap agro -industrial wastes makes their applications cost effective and increases its industrial importance further [25]. Thus, biosurfactants serve to be active weapons, being applicable as extensive tools in bioremediation technologies [26–28].

Though biodegradation has gained importance over conventional technologies, essential information about toxicity and water quality of industrial effluents has not been given much attention [29]. Assessment of ecotoxicological impact of biodegraded wastewater and phenolic components on plant system is of great concern as plants play an active role in transferring contaminants to higher trophic levels [30]. The common onion *Allium cepa*, which is regularly used, is a good bioindicator and an excellent genetic model for the screening and monitoring of toxic pollutants in the environment [31,32].

In the present investigation, we have identified and characterized a suitable microorganism from paper pulp effluent that has potential abilities to degrade *o*-cresol and reduce genotoxicity imparted by *o*-cresol, shown via root length inhibition, mitotic index (MI), and comet assay. A detailed study of kinetics of degradation and investigation of the possible degradation pathways was done. Specific enzymatic study revealed that *ortho*-cleavage pathway may play active role in degradation of *o*-cresol. Furthermore, an extensive screening to check the biosurfactant-producing abilities of the isolated microbe has been carried out followed by its extraction and characterization to establish the role of biosurfactant in cooperating with the microbe to tolerate, adapt, and degrade *o*-cresol. Thus, a single bacterial monoculture is utilized in waste (*o*-cresol) degradation as well as valorization of an industrially valuable product, i.e. biosurfactant, parallel to waste removal.

2. Materials and methods

2.1. Materials

Ortho-cresol, inorganic salts, acetonitrile, phosphoric acid, CTAB (cetyl trimethyl ammonium bromide), methylene blue (MB), iodine chips, methanol (high-performance liquid chromatography (HPLC) grade), NaCl (sodium chloride), SDS (sodium dodecyl sulfate), Triton-X 100, thin layer chromatography (TLC) silica gel plates were purchased from Merck, India. Biochemical kit, Grams staining kit, and EtBr (ethidium bromide) were from Himedia Laboratories, India. Normal melting agarose, low melting agarose, Tris buffer, and EDTA (ethylenediaminetetraacetate) were from Sigma Aldrich, USA. Kerosene and vegetable oil were purchased from local markets. All other reagents used were of analytical grade.

2.1.1. Isolation, characterization, and identification of bacteria from industrial paper pulp effluent

Industrial paper pulp effluent from Durgapur, West Bengal, India, was thoroughly screened for *o*-cresol-tolerating and *o*-cresol-degrading bacteria. Screening was done in nutrient agar and minimal salt media (MSM) agar supplemented with different concentrations of *o*-cresol (5–2,000 mg L^{-1}). The obtained bacterial isolate was characterized by gram staining and biochemical tests (IMViC

tests) [33]. Molecular identification using 16S rDNA sequence was done from Chromas Biotech Pvt. Ltd, Kolkata, India. Blastn tool analysis followed by Clustal W program with the best matched sequences were retrieved from the database [http:/ncbi.nlm.nih.gov]. The bacterial isolate is designated as PY (paper pulp yellow) since it has been isolated from paper pulp effluent and showed yellow cream colored colonies.

2.1.2. Acclimatization of bacterial isolate in o-cresol

Acclimatization of the bacterial isolate was carried out over a period of 3–6 months in MSM containing KH_2PO_4 (0.27 g L^{-1}), K_2HPO_4 (0.35 g L^{-1}), $FeCl_2{\cdot}6H_2O$ (0.002 g L^{-1}), NH_4Cl (0.53 g L^{-1}), $CaCl_2{\cdot}6H_2O$ (0.07 g L^{-1}), $MgCl_2{\cdot}6H_2O$ (0.1 g L^{-1}), $MnCl_2{\cdot}4H_2O$ (0.01 g L^{-1}), and $Na_2MoO{\cdot}_4 2H_2O$ (0.01 g L^{-1}) with gradual increase in *o*-cresol concentration (5–1,000 mg L^{-1}) as sole carbon and energy source along with regular growth monitoring.

2.1.3. Batch studies for monitoring o-cresol biodegradation with bacterial growth

Batch experiments for monitoring *o*-cresol degradation were conducted in 100 mL MSM containing different *o*-cresol concentrations (5–200 mg L^{-1}) and inoculated with 5% inoculum volume of overnight activated culture of the bacterium. The isolated bacterial monocultures were maintained in MSM (pH 7.0) at 30°C with agitation speed of 120 rpm. Bacterial growth and *o*-cresol degradation were monitored at regular intervals by spectrophotometric analysis at optical density $(OD)_{600}$ and OD_{270}, respectively. Ultra violet (UV)-Vis spectrophotometer (Agilent Technology, Cary 60) was used for the studies. Cells were harvested at 6,000× g for 20 min for biomass estimations. HPLC (Waters 600, Milford, USA) was done to further analyze *o*-cresol degradation. For estimation of *o*-cresol by HPLC [C18 column (4.6 mm × 250 mm), particle size (5 μm)], 10 μL aliquot of filtered sample (Millipore filter 0.2 μm) was injected and analyzed in UV-Vis detector (Water 2489) at 270 nm. A ratio of methanol:phosphoric acid:water (35:25:30) was used as the mobile phase with the flow rate of 1.0 mL min^{-1}.

2.1.4. Kinetic studies

Determining growth parameters is an essential criterion for designing biological reactors for microbial degradation of specific pollutants. Growth and degradation kinetics were analyzed for *o*-cresol degradation by PY where *o*-cresol is the sole source of carbon and energy.

2.1.4.1. Growth kinetics

In batch reactor, kinetic of cell growth was determined by the following equation:

$$\frac{dX}{dt} = \mu X - K_d X \tag{1}$$

where μ is the specific growth rate (h^{-1}), X is the cell growth, and K_d is the rate of decay constant (h^{-1}).

During exponential growth, K_d can be assumed to be negligible. Hence, Eq. (1) can be written as

$$\frac{dX}{dt} = \mu X \tag{2}$$

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{3}$$

Integrating above equation

$$\mu\left(t_f - t_i\right) = \ln\frac{x_t}{x_i} \tag{4}$$

$$\mu\left(t_f - t_i\right) = \ln\frac{OD_f}{OD_i} \tag{5}$$

where x_i is the initial growth of bacterial biomass, x_f is the final growth of bacterial biomass at time t, OD_i is the initial OD (at 600 nm) and OD_f is final OD (at 600 nm) at time t. The plot was made against OD versus time (t (h)), as OD is directly proportional to microbial growth. The specific growth rate was calculated from the exponential phase of microbial growth at each initial concentrations of *o*-cresol (5–1,000 mg L^{-1}).

For selecting the best fitted model(s), the experimental data were fitted to the well-known growth kinetics model(s). Two approaches were considered as per literature in the present study. According to one, the substrate is considered growth stimulatory for bacteria and represented by Monod's model [34]. The Monod's noninhibitory kinetic equation is given below:

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{6}$$

where μ is the specific growth rate (h^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}), S is the substrate concentration (mg L^{-1}), and K_s is the half saturation constant (mg L^{-1}).

In the second approach, when the substrate shows inhibitory effects on bacterial growth, different substrate inhibition models were fitted to the experimental data for selecting best model [34] (data not shown). Among them, Aiba model of substrate inhibition was represented in this study as it was fitted best to the experimental data. In 1968, Aiba et al. [35] proposed a model of substrate inhibition kinetic to express microbial growth.

The Aiba model of inhibitory growth kinetic equation is as follows:

$$\mu = \left(\frac{\mu_{max} S}{K_s} + S\right)\exp\left(\frac{-S}{K_I}\right) \tag{7}$$

where K_I is the substrate inhibition constant (mg L^{-1}) and K_S is half velocity constant (mg L^{-1}). To determine the value of μ, the exponential data of growth curve was considered.

Monon model of growth kinetics was used to calculate the values of μ_{max} and K_s, whereas the substrate inhibitory models were used to calculate K_I using nonlinear regression analysis in GraphPad PRISM® 6 software based on Windows 8.1.

2.1.4.2. Degradation kinetics

The specific degradation rate '*q*' was calculated as follows:

$$q = -\frac{1}{S}\left(\frac{dS}{dt}\right) \tag{8}$$

$$q = \frac{\left(-\ln\frac{S}{S_0}\right)}{t_f - t_i} \tag{9}$$

where S_0 is the initial substrate concentration (mg L^{-1}) at time $t = 0$ and S is the substrate concentration (mg L^{-1}) at time t_f

The present experimental data on batch degradation of *o*-cresol by *P. fluorescens* NITDPY were fitted to the available models of Halden and Aiba to select the suitable degradation kinetics models [34,36,37]. The degradation rate, q (h^{-1}), for these two models are represented in Table 1, where q_{max} is the maximum specific degradation rate (h^{-1}), K_S is the substrate affinity constant (mg L^{-1}), and K_I is the substrate inhibition constant (mg L^{-1}). To determine the degradation rate q, S vs. time for each initial substrate concentration is plotted in a semi-logarithm plot. By using nonlinear regression analysis in GraphPad PRISM 6 based on Windows 8.1, from the plot of q vs. $-\ln S/S_0$, the values of the kinetic parameters for those models were obtained.

2.1.5. Enzyme assays

P. fluorescens NITDPY grown in MSM supplemented with 200 mg L^{-1} of *o*-cresol was harvested, centrifuged at 6,000× g for 10 min at 4°C, washed thrice with PBS (phosphate buffer saline, pH = 7.5), and disrupted by sonication using Sartorious LABSONIC^R M with 10S pulses and 5S gaps for 25 min in ice. The disrupted cells were centrifuged to remove cell debris, and the clear supernatant is used as crude extract of enzyme to determine the presence of catechol 1,2-dioxygenase (C12D) and catechol 2,3-dioxygenase (C23D) [38] as well as estimation of protein by Lowry method [39]. The activities of C12D and C23D in the crude extract were monitored spectrophotometrically (Agilent Technology, Cary 60) following formation of *cis*,*cis*-muconic acid (at 260 nm) and 2-hydroxymuconic semialdehyde (at 375 nm), respectively. In brief, the reaction mixture contained 20 μL of catechol (50 mM), 67 μL of EDTA (20 mM), PBS (50 mM, pH 7.5), and crude extract (50–200 μL) in a final volume of 1 mL. Prior to the determination of catechol 1,2-dioxygenase activity, 5% H_2O_2 was added to inactivate catechol 2,3-dioxygenase.

2.1.6. Screening and characterization of biosurfactant production of the bacterial isolate

The isolated bacterium was screened for the production of biosurfactants by specific assays. BATH (bacterial adhesion to hydrocarbons) assay was performed as described by Thavashi et al. [40], with modification. BATH index was calculated using the formula give below.

$$\text{BATH index} = \left(\frac{1 - \text{OD after treatment}}{\text{OD before treatment}}\right) \times 100$$

Emulsification assay was done as described by Rajamani et al. [41]. To check the emulsifying activity of PY, *E. coli* was taken as negative control. *E. coli* cell supernatant and MSM were used as negative control. The emulsification index (E_{24}) was calculated using the formula:

$$\text{Emulsifying index}\left(E_{24}\right) = \left(\frac{\text{Height of emulsified layer}}{\text{Height of column}}\right)$$

$$\%\ \text{Emulsification} = E_{24} \times 100.$$

Foam height assay was done as described by El Sheshtawy et al. [42]. Foam percentage was calculated using the formula:

$$\left(\frac{\text{Foam height}}{\text{Height of the column}}\right) \times 100 = \text{Foaming \%}$$

Surface tension was measured by Ring Method (Lauda CD-1C).

Production of biosurfactant was also assessed via microplate grid assay, drop collapse assay, penetration assay, CTAB MB agar assay, and oil spreading assay according to standard protocol [22,41–44].

For biosurfactant extraction, bacteria was grown in MSM with 2% glucose as carbon source at 30°C with 150 rpm

Table 1
Degradation kinetic parameters for *o*-cresol using various models

Model	Equation	q_{max} (h^{-1})	K_s (mg L^{-1})	K_I (mg L^{-1})	R^2
Aiba	$q = \frac{q_{max}S}{K_s + S}\exp\left(\frac{-S}{K_I}\right)$	0.0731	80.16	260.07	0.9748
Haldane	$q = \frac{q_{max}S}{K_s + S + \left(\frac{S^2}{K_I}\right)}$	0.0554	50.50	103.6	0.8967

Specific degradation rate (q, h^{-1}) of the isolated bacteria in different concentrations of *o*–cresol (50–800 mg L^{-1}) was calculated and fitted in different degradation kinetic models for calculation of kinetic parameters and regression coefficient.

agitation for 72 h. Cells were harvested at 7,000 rpm for 15 min. Cell-free culture supernatants were acidified to pH 2 and were allowed to stand overnight at 4°C for acid precipitation [45]. These were centrifuged at 13,000 rpm for 15 min to obtain precipitates of the surfactants. pH was adjusted to physiological pH and extracted by chloroform:methanol (2:1) method and air dried to obtain crude surfactant [46,47]. Extracted biosurfactant was characterized by TLC and Fourier transform infra-red spectroscopy (FTIR). TLC using chloroform:methanol:acetic acid (81:17:2) ratio as mobile phase on silica 60 gel aluminium plates was used (Merck, Dermstadt, Germany) [22]. Spots were developed using iodine vapors and seen under UV transilluminator.

FTIR was conducted using the crude biosurfactant extracted and analyzed for specific functional groups using Therm Nicolet iS10 spectrometer following Donio et al. [48].

2.1.6.1. The effect of o-cresol on biosurfactant production

Bacterial isolate was grown overnight in MSM at 30°C with 120 rpm agitation. Activated culture of PY was used to inoculate in 100 mL MSM with glucose (2%) as carbon source in the presence of different concentrations of *o*-cresol (25–250 mg L^{-1}). These were incubated at 30°C with agitation of 120 rpm for 72 h. Cells were then centrifuged, and clear cell-free supernatant was collected. Emulsification assay was conducted. Bacterial biomass was measured for each set of batch culture by harvesting cells at 6,000 g for 15 min and drying the pellets at 70°C overnight in hot air oven. Crude surfactant was dried and weighed after extraction by chloroform methanol method.

2.1.7. Ecotoxicological analysis

2.1.7.1. Root growth inhibition and MI test

Allium cepa root growth inhibition test was performed as stated by Fiskesjo 1985 [49] with slight modification. Onion bulbs (diameter, 7.5 mm × 8.5 mm; weight, 2.5–3.5 g) were purchased from local market, and the dead outer scales and dry bases were removed to expose the root primordial. Prior to treatment with the test reagents, root initiation was done by exposing to tap water for 48 h. *O*-cresol of 10and 200 mg L^{-1}, untreated and bacterial treated, was added separately to onion bulbs and incubated for 24 h in plant growth chamber maintaining proper condition. The root lengths of the onion bulbs were measured at each point of time. Following general protocol, fixation and staining of the root tip cells were done to evaluate MI and frequencies of chromosomal aberrations (CA) [50].

2.1.7.2. Comet assay

Single-cell gel electrophoresis assay (comet assay) was performed to determine potential genotoxic effects of *o*-cresol on *A. cepa* before and after the specific bacterial treatment. The onion bulbs were processed for the comet assay following the method described by Singh et al. and Yıldız et al. [51,50]. About 20 mg of meristematic root tips of onion bulbs were chopped using a razor blade in ice cold Tris-$MgCl_2$ buffer (pH 7.5), filtered through nylon net (52 μm), and centrifuged to isolate the root nuclei. The nuclear suspension of 100 μL was mixed with 0.8% low-melting agarose (100 μL) at 37°C. It was then dropped on a precoated slide containing 1% normal melting agarose and covered with cover slips to form a uniform layer. The slides were kept on ice tray for 2 min, and the cover slips were removed and immersed in ice cold lysis buffer (pH 12.3) for 1 h. Subsequently, the slides were placed in electrophoretic buffer (pH 12.3) for 30 min for unwinding of DNA, and electrophoresis was conducted for 20 min at 25 V (1 V cm^{-1}). The slides were washed with neutralization buffer (400 mM Tris, pH 7.5) every 5 min followed by EtBr (20 μg mL^{-1}) staining for 5 min. Excess stain was removed by washing with cold water and analyzed under fluorescent microscope (Dewinter Premium, Italy). The captured images were analyzed with comet analysis software CASP-1.2.3b1 (http://casp.sourceforge.net/index.php). For each treatment condition, 50 randomly chosen nuclei from three slides were analyzed using fluorescent microscope for tail length (TL, μm), percent tail DNA (%TDNA), and olive tail moment (OTM). Each image was classified according to the intensity of the fluorescence in the comet tail-assigned value of either 0, 1, 2, 3, or 4 so that the total scores of slide could be between 0 and 400 arbitrary units (AU) [52].

2.1.8. Statistical analysis

All batch experiments were carried out at 30°C ± 2°C with triplicate. The presented results are mean values ± standard deviation from three independent experiments.

3. Results

3.1. Characterization and identification of O-cresol-resistant bacterial strain

Tolerance of the isolated species to *o*-cresol via minimum inhibitory concentration obtained was 1,500 mg L^{-1}. Biochemical test of the Gram-negative bacteria revealed it as catalase, indole, methyl red, urea, casein, and oxidase positive as represented in Table 2. The 16S rDNA sequence and corresponding phylogenetic tree of the isolated PY showed 99% close homology with *P. fluorescens* strain CR2 (Fig. 1). The partial nucleotide sequence of this bacterium containing 1,458 bp has been deposited to GenBank, and accession no. KM111571 has been assigned to this strain. From the above findings, this *o*-cresol-resistant bacterial strain has been designated as *Pseudomonas fluorescens* NITDPY in the present study.

3.2. Bacterial growth, degradation of o-cresol, and its kinetics

3.2.1. Effect of various concentrations of o-cresol on growth

The specific growth rate of the bacterial isolate increased efficiently up to 200 mg L^{-1} with *o*-cresol as the only source of carbon and energy, and the maximum specific growth rate (μ_{max}) was 0.1804 h^{-1}. The experimental data for the concentration range of 5–200 mg L^{-1} *o*-cresol fitted well with the Monod model with a correlation coefficient (R^2) of 0.9431, μ_{max} of 0.3186 h^{-1}, and K_s of 133.8 mg L^{-1}. Fig. 2 depicts that for higher concentrations of *o*-cresol (200–1,000 mg L^{-1}), growth declined, showing inhibitory nature of *o*-cresol. Aiba model fitted best with correlation coefficient R^2 of 0.9460, μ_{max} (0.4886 h^{-1}), K_S (131.3 mg L^{-1}), and K_I (410.84 mg L^{-1}) (Table 3).

Table 2
Morphological, physiological, biochemical and molecular characterization of paper pulp effluent-isolated bacterium *P. fluorescens* NITDPY with sugar utilization phenomenon

Morphological/physiological/ biochemical/molecular characteristics/sugar utilization	Isolated bacterium PY
Gram stain	–
Cell shape	Bacilli
Characteristics in Agar plate	Soft, smooth, yellow
Growth at temperature (°C)	30
Indole	–
Methyl Red(MR)	+
Voges-Proskauer (VP)	–
Citrate utilization	–
Glucose	+
Adonitol	–
Arabinose	–
Lactose	–
Sorbitol	–
Manitol	–
Rhamnose	–
Sucrose	+
Phenol (mg L^{-1})	500
Catechol (mg L^{-1})	300
2,4,6-trichlorophenol (mg L^{-1})	600
16S rDNA	*Pseudomonas fluorescens* NITDPY

+ = positive, – = negative.

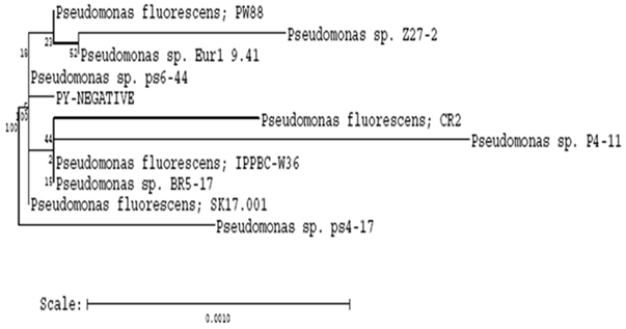

Fig. 1. Phylogenetic analysis of paper pulp-isolated bacterial monoculture *Pseudomonas fluorescens* NITDPYKM111571.

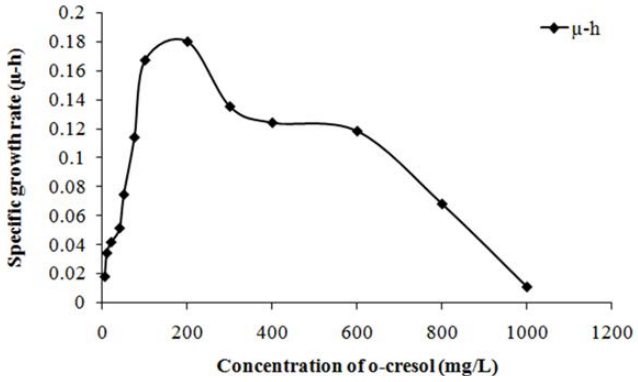

Fig. 2. Specific growth rate of *P. fluorescens* NITDPY in MSM supplemented with different initial concentrations of *o*-cresol (5–1,000 mg L^{-1}) as sole source of carbon at 30°C, pH 7, with continuous agitation (120 rpm).

3.2.2. Degradation of o-cresol and its kinetics

Bacterial growth was significantly fair at lower concentrations of *o*-cresol (5–20 mg L^{-1}) with almost 100% degradation after 72 h of treatment. The percent *o*-cresol degradation of 85.9 ± 0.12, 82.1 ± 0.33, 80.3 ± 0.23, 57.6 ± 0.11, 44.5 ± 0.41, 31.6 ± 0.21, 25.6 ± 0.31, and 14.5 ± 0.22 at 50, 100, 200, 300, 400, 600, 800, and 1,000 mg L^{-1}, respectively, was obtained. The degradation of 50, 100, and 200 mg L^{-1} of *o*-cresol is significantly high. In contrast, at higher concentrations, the percent degradation reduced significantly. Fig. 3 shows correlation between the growth of *P. fluorescens* NITDPY and its potential in degrading 200 mg L^{-1} of *o*-cresol in batch study at optimized condition.

For degradation kinetic study, in Fig. 4, a typical plot on negative log of S/S_0 vs. time gave a straight line indicating the rate of degradation q to be first order with the value of 0.025 h^{-1} at *o*-cresol concentration of 200 mg L^{-1}. Aiba model fitted well to the experimental data with correlation coefficient 0.9748 and q_{max} (0.0731 h^{-1}), K_s (80.16 mg L^{-1}), and K_I (260.07 mg L^{-1}). Table 4 shows comparison of growth kinetic

Table 3
Growth kinetic parameters for *o*-cresol using various models

Model	Equation	μ_{max} (h^{-1})	K_s (mg L^{-1})	K_I (mg L^{-1})	R^2
Monod model	$\mu = \frac{\mu_{max} S}{K_s + S}$	0.3186	133.8	–	0.9431
Aiba	$\mu = \frac{\mu_{max} S}{K_s + S} \exp\left(\frac{-S}{K_I}\right)$	0.488	131.3	410.84	0.9460

Specific growth rate (μ, h^{-1}) of the isolated bacteria in different concentrations of *o*-cresol was calculated and fitted in different growth kinetic models for calculation of kinetic parameters and regression coefficient. Monod model fitted to lower range (5–200 mg L^{-1}) and the growth inhibitory model Aiba fitted to higher range (200–1,000 mg L^{-1}) *o*–cresol.

parameters for biodegradation of *o*-cresol by mixed and pure bacterial cultures.

3.3. Study of enzyme involved in degradation

The activities of catechol 1,2-dioxygenase (C12O) and catechol 2,3-dioxygenase (C23O) were examined in the crude extract of the isolated strain *P. fluorescens* NITDPY in the presence of *o*-cresol (200 mg L^{-1}). For C12O assay, the OD of reaction mixture at 260 nm increased as a function of time indicating the formation of *cis-cis* muconic acid, whereas negligible enhancement at $OD_{375\ nm}$ was observed. The specific activity of C12O was 2.404 ± 0.0527 mmol min^{-1} mg^{-1} protein in 72 h treatment set as compared with 0.904 mmol min^{-1} mg^{-1} C12O in control. The activity of C23O of 0.700 mmol min^{-1} mg^{-1} protein appeared negligible in the same experimental set.

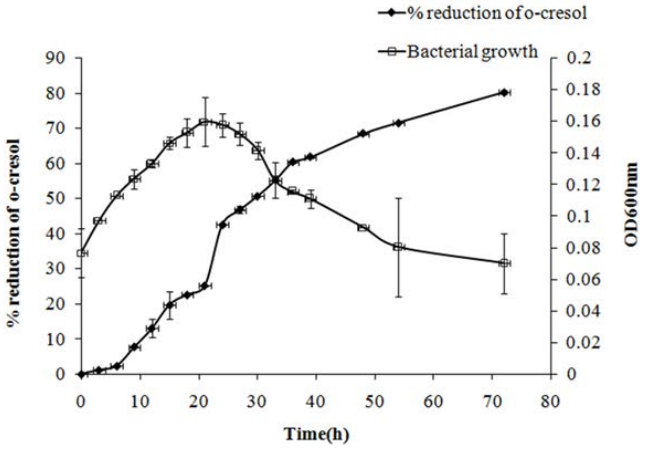

Fig. 3. Correlation between the growth of *P. fluorescens* NITDPY and its potential in degradation of 200 mg L^{-1} of *o*-cresol in batch study at optimized condition.

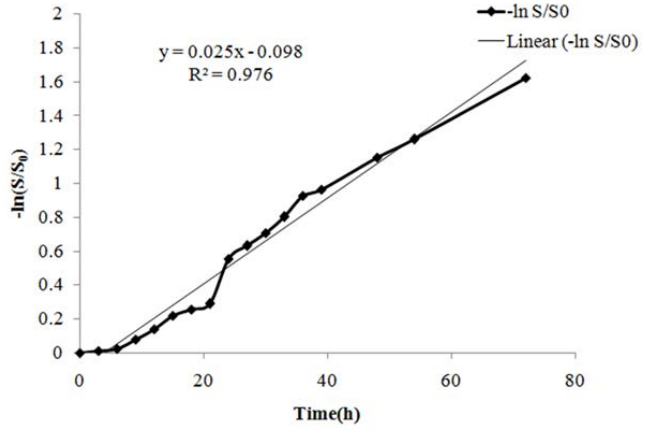

Fig. 4. Plot between time (h) versus $-\ln(S/S_0)$ for determination of q at the initial *o*-cresol concentration of 200 mg L^{-1}.

3.4. Biosurfactant and its role in o-cresol degradation

3.4.1. Screening and characterization

All qualitative assays to confirm the production of biosurfactant showed positive results as shown in Table 5. BATH index and emulsification index (E_{24}) were calculated to be 63.2 ± 3.5 and 35 ± 5. The cell-free supernatants yielded a good foaming height (Fig. 5(a)) of 64.5% ± 2.5%. Microplate grid assay, penetration assay, drop collapse assay, and oil spreading assay (Fig. 5(b)) showed very good results for PY. All the assays showed no activity in *E. coli*. CTAB MB agar assay (Fig. 5(c)) showed observable changes in wells containing PY as compared with distilled water as negative control and 2% SDS as positive control. Cell-free supernatants lowered the surface tension of water to 36 mN m^{-1}.

Results of TLC showed proper spots with Rf values of 0.74 and 0.36. FTIR analysis in Fig. 6 shows significant peaks at 3,400, 1,636.96, and a slight stretch at 1,077.26.

Table 5
Assays for screening potential of biosurfactant production for *P. fluorescens* NITDPY

Assays for screening of BS production	*P. fluorescens* NITDPY	*Escherichia coli*
BATH	++++	–
Emulsification	++++	–
Microplate grid	++++	–
Drop collapse	+++	–
Penetration assay	+++	–
CTAB blue agar plate	+++	–
Oil spreading assay	+++	–

+++ = very good response; ++++ = excellent response; – = no detectable response.

Table 4
Comparison of growth kinetic parameters for biodegradation of *o*-cresol by mixed and pure bacterial culture

Authors	Bacterial strain	μ_{max} (h^{-1})	K_s (mg L^{-1})	K_I (mg L^{-1})	Kinetic model
Kar et al., (1997)	*Arthrobacter* sp. MTCC 1,553	0.84	84	1,050	Andrew
Ho et al., (2010)	Mixed culture	2.78	15.3	192	Andrew
Maeda et al., (2005)	Mixed culture (medium A)	0.37	92.4	125	Andrew
Maeda et al., (2005)	Mixed culture (medium B)	0.11	2.8	325	Andrew
Lassouane et al., (2013)	*Pseudomonas aeruginosa* S8	0.335	246	304.2	Andrew
Kaymaz et al., (2012)	*P. putida* 548 (pJP4)	0.519	130.883	223.84	Haldane
Present study	*P. fluorescens* NITDPY	0.488	131.3	410.84	Aiba

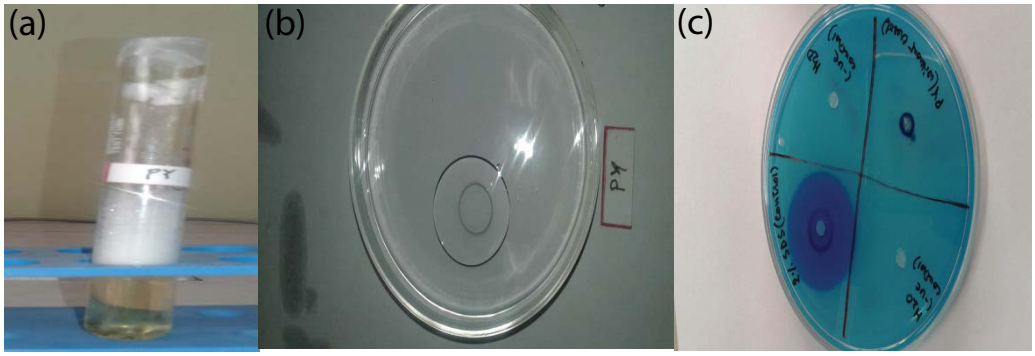

Fig. 5. Qualitative assays for biosurfactant from *P. fluorescens* NITDPY. (a) Foaming height assay, (b) Oil displacement test, (c) CTAB MB agar assay.

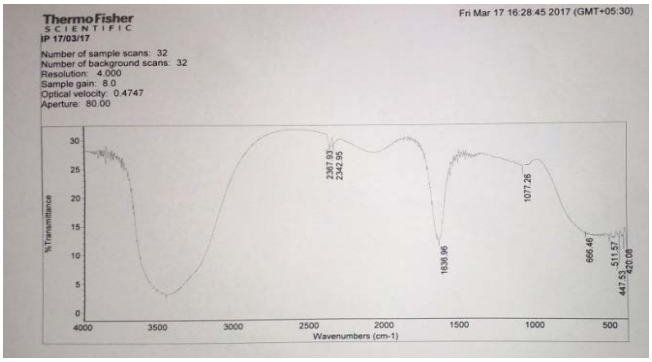

Fig. 6. FTIR of crude biosurfactant extracted from *P. fluorescens* NITDPY.

3.4.2. Effect of o-cresol on biosurfactant production

The emulsification index showed a significant increase of approximately 1.86 fold (from 35% to 64%), and dry biosurfactant yield was increased approximately 2.5 fold in the presence of *o*-cresol induction in a wide range of concentrations (25–250 mg L^{-1}) (Table 6). Percent *o*-cresol removal from the media in batch experiment showed about 93.5 ± 0.2, 85.9 ± 0.01, 84.1 ± 0.32, 82.1 ± 0.03, 80.3 ± 0.23, and 75.8 ± 0.2 for 25, 50, 80, 100, 200, and 300 mg L^{-1} of *o*-cresol, respectively, i.e. the percent degradation varied by only 3%–1% (Table 6).

3.5. Eco-safety assessment of bacterial treatment

3.5.1. Root growth inhibition and chromosomal abnormalities in Allium cepa (A. cepa) cells

The observed root lengths of *A. cepa* in bacterial-treated 10 and 200 mg L^{-1} *o*-cresol were 2.44 ± 0.19 cm and 1.86 ± 0.15 cm compared with untreated sample (1.37 ± 0.16 cm and 1.27 ± 0.18 cm). The percent root growth enhancements in treated 10 and 200 mg L^{-1} *o*-cresol were 79 ± 5 and 61 ± 4 compared with untreated samples. The decrease in MI of *A. cepa* root meristematic cells (Table 7) exposed to untreated 10 and 200 mg L^{-1} *o*-cresol (3.7 ± 0.17 and 1.7 ± 0.13) compared with tap water as control was observed (20 ± 02). However, there is increase in MI of *A. cepa* meristematic cells when exposed to bacterial-treated *o*-cresol at the same concentrations (9.2 ± 0.11 and 6.5 ± 0.19).

3.5.2. Comet assay

3.5.2.1. Analysis of A. cepa root meristem exposed to untreated and PY-treated o-cresol

The results summarized in Table 8 show that as compared with positive (0.2% w/v ethyl methane sulfonate) and negative controls (tap water), the DNA damage was significantly high in 200 mg L^{-1} untreated *o*-cresol, whereas bacterial-treated *o*-cresol showed less DNA damage. The highest DNA damage was observed in *A. cepa* root nuclei in the presence of *o*-cresol compared with positive and negative controls (Fig. 7). From Table 8, it is observed that *A. cepa* exposed to PY-treated *o*-cresol (200 mg L^{-1}) showed 3.6, 5.0, and 7.0 fold decrease in %TDNA, TL, and OTM, respectively, compared with its untreated control.

4. Discussion

The results obtained from the current study have unwrapped a significant divergent nature of the bacterial isolate *P. fluorescens* NITDPY. Experiments conducted not only showed *Pseudomonas fluorescens* NITDPY to be a potent degrader of *o*-cresol but is also detected to be producing a worthy bacterial product like biosurfactant simultaneously. *P. fluorescens* NITDPY has also shown remarkable calibre to grow in other phenolic components, viz., phenol (500 mg L^{-1}), catechol (300 mg L^{-1}), and 2,4,6-trichlorophenol (600 mg L^{-1}), besides *o*-cresol, hence finding better applicability in environmental pollution cleanup. In the present investigation, the isolated bacterial strain, *P. fluorescens* NITDPY was acclimatized in *o*-cresol for specific enzyme induction that plays pioneer roles in the degradation metabolism of toxic phenolic components.

The effect of *o*-cresol on the growth of *P. fluorescens* NITDPY imparted interesting results where we observed that *P. fluorescens* NITDPY can utilize *o*-cresol as the sole source of carbon and energy. The specific cell growth rate evaluated for each *o*-cresol concentration showed that the specific growth rate increased efficiently up to 200 mg L^{-1} with the maximum specific growth rate (μ_{max}) of 0.1804 h^{-1}, suggesting *o*-cresol to be promoting its growth up to 200 mg L^{-1} (Fig. 2). Specific growth rate declined beyond this range. Similar phenomenon has been reported in literatures [2,53,54]. The values of the different growth kinetic parameters obtained based on

Table 6
Effect of *o*-cresol on biosurfactant production and corresponding *o*-cresol degradation percent

O-cresol concentration (mg L^{-1})	Emulsification percent	Biomass (g)	Dry surfactant weight (g)	*O*-cresol degradation (%)
0	34	0.28	0.086	–
25	63.5	0.305	0.215	93.5 ± 0.2
50	63.5	0.311	0.220	85.9 ± 0.01
80	64	0.29	0.205	84.1 ± 0.32
100	64.5	0.288	0.200	82.1 ± 0.03
200	63.5	0.3	0.210	80.3 ± 0.23
300	63	0.27	0.198	75.8 ± 0.2

Table 7
Root length inhibitions, chromosomal aberration, and mitotic index of *A. cepa* root cells exposed to untreated and 24-h *P. fluorescens* NITDPY-treated *o*-cresol

Test samples	Average root length (cm) Mean ± SD	Root growth (%)	Root inhibition (%)	Chromosomal aberration (%)	Mitotic index (%)
Negative control (tap water)	3.08 ± 0.24	100 ± 11	0	4.9 ± 0.87	20.4 ± 2
Negative control (0.2% v/v EMS)	3.06 ± 0.2	–	–	75.39 ± 03	3.1 ± 0.01
Untreated o-cresol (10 mg L^{-1})	1.37 ± 0.16	45 ± 3	55 ± 2	63.72 ± 1.87	3.7 ± 0.17
24-h P. fluorescens NITDPY-treated 10 mg L^{-1} o-cresol solution	2.44 ± 0.19	79 ± 5	21 ± 3	28.12 ± 1.92	9.2 ± 0.11
Untreated o-cresol (200 mg L^{-1})	1.27 ± 0.18	41 ± 7	59 ± 5	82.47 ± 2.09	1.7 ± 0.13
24-h P. fluorescens NITDPY-treated 200 mg L^{-1} o-cresol solution	1.86 ± 0.15	61 ± 4	39 ± 2	40.36 ± 1.73	6.5 ± 0.19

Mean ± = standard deviation of three individual experiments, EMS = ethyl methane sulfonate.

Table 8
Detection of DNA damage in nuclei of *Allium cepa* root meristem following 24 h exposure to untreated and 24-h *P. fluorescens* NITDPY-treated *o*-cresol (200 mg L^{-1}) using comet assay

Types of sample	Tail DNA (%) Mean ± SD	Tail length (μm) Mean ± SD	Olive tail moment (AU) Mean ± SD
Tap water (negative control)	1.64 ± 0.93	1.9 ± 0.19	0.36 ± 0.005
EMS (positive control)	6.17 ± 1.85	61.68 ± 22.41	3.01 ± 0.98
Untreated o-cresol (200 mg L^{-1})	22.36 ± 3.32	211.60 ± 61.18	15.23 ± 3.51
P. fluorescens NITDPY-treated o-cresol (200 mg L^{-1})	6.92 ± 1.98	43.39 ± 21.22	2.38 ± 0.71

Mean ± = standard deviation of three individual experiments, EMS = ethyl methane sulfonate.

the fitting of various models for *o*-cresol-mediated growth and biodegradation by P. fluorescens NITDPY along with a comparison of previously reported data are shown in Table 4.

A detailed kinetic study was done to observe the effect of *o*-cresol on growth along with its degradation. Monod model of growth kinetics fitted best with stimulatory effect of substrates as per previous reports [34]. In the present investigation, the experimental data for concentration range of 5–200 mg L^{-1} *o*-cresol fitted well with the Monod model with a correlation coefficient (R^2) of 0.9431, μ_{max} of 0.3186 h^{-1}, and K_s of 133.8 mg L^{-1}. In Monod model, K_I is not applicable in this case since it is a noninhibitory unstructured model. K_s calculated from Monod model does not represent the true picture when effect of inhibitory substrate on microbial growth was considered. Fig. 2 depicts that for higher concentrations of *o*-cresol (200–1,000 mg L^{-1},), growth declined, showing the inhibitory nature of *o*-cresol. Therefore, different inhibitory models were used to calculate K_I value. A variety of substrate inhibition models known have been successfully used to describe the dynamics of microbial growth on toxic phenolic components [6,7,37,55].

Different kinetic models were fitted to the experimental data in order to determine the kinetic parameters, viz., μ_{max}, K_s, and K_I. Aiba model fitted best with correlation coefficient (R^2) of 0.9460, μ_{max} (0.4886 h^{-1}), K_s (131.3 mg L^{-1}), and K_I (410.84 mg L^{-1}) (Table 1). The values from this model intimate that *P. fluorescens* NITDPY could grow well at high concentrations of *o*-cresol. The K_I value of 410.84 mg L^{-1}

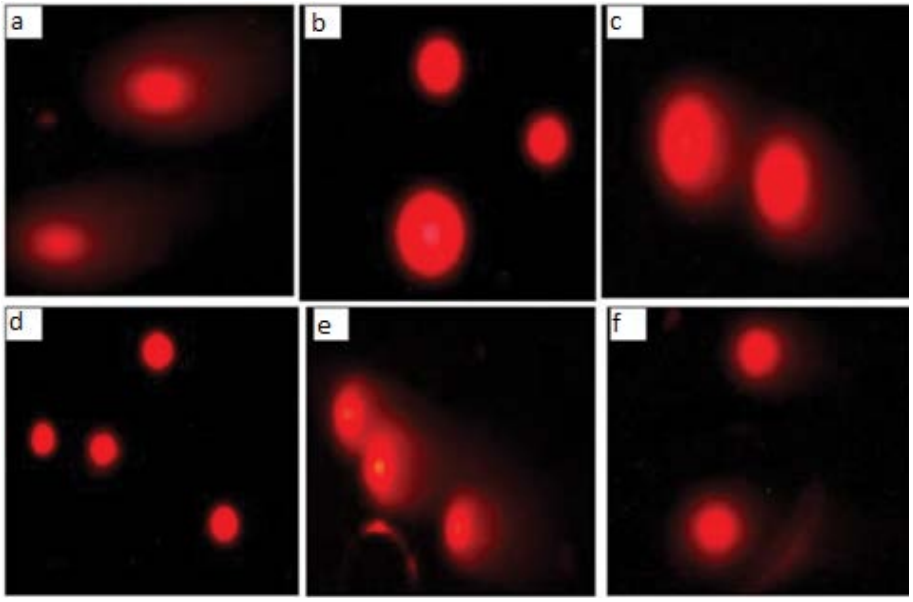

Fig. 7. Photomicrograph of root nuclei-based comet observed on *A. cepa* exposed to untreated and 24-h *P. fluorescens* NITDPY-treated *o*-cresol (10 and 200 mg L^{-1}). (a) Positive control (0.2% w/v EMS), (b) Negative control (tap water), (c) Untreated *o*-cresol (10 mg L^{-1}), (d) Treated *o*-cresol (10 mg L^{-1}), (e) Untreated *o*-cresol (200 mg L^{-1}), (f) Treated *o*-cresol (200 mg L^{-1}).

corroborates that treatment process by this isolated bacterial monoculture can be effective at high concentrations of *o*-cresol. The growth kinetic parameters in phenolic waste compound studied here correlate with the past reports on *Pseudomonas* sp. [2,8,54]. Table 4 represents the comparison of reported kinetic parameters of different microorganisms with the present study on the biodegradation of *o*-cresol. Though μ_{max} obtained in this study is comparable with other reported bacterial species [2,6,56,57], K_I value was found higher than the reported value for *o*-cresol degradation by bacteria of same genera indicating high resistance to the substrate [2,54]. This emphasizes that such high resistant indigenous bacterial isolate could be useful in the degradation of phenolic wastes of industrial origin.

Significantly higher degradation of 50, 100, and 200 mg L^{-1} of *o*-cresol is contrasting with that of the higher concentrations of *o*-cresol where the percent degradation reduced observably. Previous reports suggested that percent degradation can decrease at higher concentrations of phenolic components [36,37]. This observation could be well explained with the specific growth rate of *P. fluorescens* NITDPY at various concentrations of *o*-cresol studied as shown in Fig. 2. The correlation study at 200 mg L^{-1} in Fig. 3 depicts a positive correlation between the growth and percent degradation.

The negative log of S/S_0 versus time was plotted for degradation kinetic studies which resulted in a straight line, thus depicting the rate of degradation q to be in first order with the value of 0.025 h^{-1} at *o*-cresol concentration of 200 mg L^{-1}. The degradation rate can be correlated with phenol degradation rate by other reported bacterial strains [58]. The degradation rate at different initial concentrations of 100–1,000 mg L^{-1} fitted with kinetic models using nonlinear regression analysis in GraphPad PRISM 6 to assess the kinetic behavior of *o*-cresol degradation by *P. fluorescens* NITDPY. The results suggest that Aiba model fitted well to the experimental data with correlation coefficient of 0.9748 and q_{max} (0.0731 h^{-1}), K_s (80.16 mg L^{-1}), and K_I (260.07 mg L^{-1}).

The mechanism involved in bacterial *o*-cresol degradation is less studied as compared with well-documented pathways involved in phenol degradation. Since the isolated strain efficiently degraded *o*-cresol, the pathway involved in *o*-cresol degradation was explored. The specific activity of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase disclosed that the latter is negligible when compared with the former. It was reported that *o*-cresol degradation by certain species of *Pseudomonas* was initiated by the formation of 3-methylcatechol, catechol being an important intermediate formed during the degradation of phenolic components [2]. In the next step, two enzymes can be induced, catechol 1,2-dioxygenase or catechol 2,3-dioxygenase, which belong to the *ortho* and *meta* ring fission pathways, respectively [10]. It is well documented that the aerobic or anaerobic degradation of phenolic compounds by different bacterial strains of *Pseudomonas* genus follows either *ortho-* or *meta*-cleavage pathway [11,12]. The present investigation showed *o*-cresol-induced C12O activity and thus hinted to follow *ortho*-cleavage pathway.

Although reports have already shown the role of biosurfactants in the degradation of phenol, bisphenol, and hydrocarbons [15], yet the role of different phenol-based polluting agents in inducing biosurfactant production has still remained unexplored. In order to check the ability of the bacterial isolate to produce biosurfactants, specific assays were conducted where all qualitative assays yielded positive results (Table 5). BATH index and emulsification index were calculated to be 63.2 ± 3.5 and 35 ± 5, respectively, thus suggesting *P. fluorescens* NITDPY can generate biosurfactants. Hydrophobicity in similar range (65%) by *Acetobacter beijerinckii* was reported by Rajamani et al. [41].

Emulsification activity by *Pseudomonas aeruginosa* was reported by Moussa et al. [59], where results resembled our study. Cell-free supernatants lowered the surface tension of water to 36 mN m^{-1}. *Pseudomonas aeruginosa* is reported to reduce the surface tension of water to 46 mN m^{-1} [23].

TLC and FTIR analyses were carried out for the crude biosurfactant extracts where TLC spots with Rf values of 0.74 and 0.36 corroborated with previous reports of di- and mono-rhamnolipids from *Pseudomonas aeruginosa*, respectively [Bhat et al. 2015]. FTIR analysis (Fig. 6) results with significant peaks at 3,400, 1,636.96, and 1,077.26 correspond to hydrophilic (O–H) bonds, hydrophobic (C=C) bond, and slight rhamnose stretch, respectively. FTIR of crude biosurfactant obtained from *Bacillus* sp. BS3 by Donio et al. [48] and *Pseudomonas aeruginosa* by Bezza et al. [60] showed similar peaks.

Prominent increase of emulsification index of 1.86 fold (from 35% to 64%) and increase in the yield of dry biosurfactant of 2.5 fold in the presence of *o*-cresol induction in a wide range of concentrations (25–250 mg L^{-1}) correlated with the percent *o*-cresol removal from the media in batch experiment showing about 93.5 ± 0.2, 85.9 ± 0.01, 84.1 ± 0.32, 82.1 ± 0.03, 80.3 ± 0.23, and 75.8 ± 0.2 for 25, 50, 80, 100, 200, and 300 mg L^{-1} of *o*-cresol, respectively, where degradation varied by 3%–1%.(Table 6). This observation is thus a new insight in biotreatment of phenolic wastes with concurrent yield of biosurfactant as a valuable product.

In environmental biomonitoring studies, *Allium* test was conducted with root growth, MI, and CA used as parameters to determine the level of cytotoxicity and genotoxicity of the pollutants [50]. To investigate the environmental applicability of *P. fluorescens* NITDPY-mediated *o*-cresol degradation, phytotoxicity on *A. cepa* was analyzed. Chauhan et al. [61] reported that the inhibition in MI can be attributed to the effect of environmental chemicals on DNA/protein synthesis of the biological system. Table 7 depicts that the isolated strain-treated *o*-cresol showed more root growth compared with its untreated control, thereby indicating reduced cytotoxicity. Further, the percent root growth enhancement in treated as compared with untreated samples indicated that the isolated bacterial strain-mediated *o*-cresol biodegradation did not generate any toxic intermediate. The observed decrease in MI of *A. cepa* root meristematic cells (Table 7) in untreated *o*-cresol samples using tap water as control indicates the mito-depressive nature of *o*-cresol. However, there is increase in MI of *A. cepa* meristematic cells when exposed to bacterial-treated *o*-cresol at similar concentrations, indicating that the antimitotic activity and mito-depressive nature of *o*-cresol diminished after the bacterial treatment. Genotoxicity of the pollutant is related to the extent of the CA [61]. Results depicted that with bacterial-treated *o*-cresol, there is about 50% reduction of CA in *A. cepa* compared with untreated one. These suggest that cytotoxic and genotoxic potential of *o*-cresol did not increase. Hence, the potential of isolated bacterial strain *P. fluorescens* NITDPY mediated degradation of this priority pollutant, without enhancing the cytotoxicity and genotoxicity in *A. cepa*. Thus, the bacterial treatment can be an environment-friendly approach towards lowering risks imposed by the toxic pollutant *o*-cresol.

The comet assay is widely accepted to determine the genotoxic hazard on a single-cell level in the root nuclei of various plant species which allows the detection of single- and double-strand breaks, incomplete excision repair sites, and cross-links [50,62]. Comet assay was done to evaluate the effect of the bacteria on DNA damage caused by *o*-cresol. Three comet assay parameters, namely, %TDNA, TL, and OTM were used for analysis of DNA damage using comet scoring program CASP-1.2.3b1 (http://casp.sourceforge.net/index.php). The highest DNA damage in *A. cepa* root nuclei in the presence of *o*-cresol (Fig. 7) might be explained with an increase in activities of both free radicals and reactive oxygen species in *o*-cresol-exposed cells. Rucinska et al. [62] suggested that at higher concentrations of environmental pollutants, the formation of both free radicals and reactive oxygen species is beyond the capacity of the antioxidant systems of the living system and thus contribute to oxidative DNA modification. Table 8 reveals that *A. cepa* exposed to *P. fluorescens* NITDPY-treated *o*-cresol (200 mg L^{-1}) showed a significant 3.6, 5.0, and 7.0 fold decrease in %TDNA, TL, and OTM, respectively, compared with its untreated control. The significant decrease in DNA damage in bacterial-treated *o*-cresol in the present study might be due to reduction in reactive species formation as well as oxidative stress in *A. cepa* root nuclei (Table 8, Fig. 7). The observations of the present study prompted us to suggest that the biodegradation of *o*-cresol by this indigenous pure culture *P. fluorescens* NITDPY could be valuable for environmentally safe biotreatment of industrial waste.

5. Conclusion

A potent indigenous bacterial isolate from paper pulp effluent identified to be *Pseudomonas fluorescens* NITDPY (KM111571) was found to be an efficient *o*-cresol degrader along with being a producer of a valorizable bacterial metabolite, i.e., biosurfactant. This strain possesses high tolerance to toxic *o*-cresol (1,000 mg L^{-1}) and is capable of degrading maximum 80.3% ± 0.23% of *o*-cresol from 200 mg $L^{-1}$of aqueous solution in 72 h of treatment. Monod model of growth kinetics fitted well to the experimental data when *o*-cresol (5–200 mg L^{-1}) was stimulatory to growth, whereas Aiba model was found correlating well with the experimental data when *o*-cresol was inhibitory to growth. Mechanistic pathway imparted involvement of *o*-cresol degradation via *ortho*-cleavage. The toxicity assessment studies post-biotreatment showed reduction of both genotoxicity and cytotoxicity of *o*-cresol on *A. cepa*, indirectly stating that there was no toxic intermediate generation during the biodegradation process. Due to the presence of biosurfactant-producing potential, bioaccumulation of pollutants like *o*-cresol is enhanced which further helps in the degradation phenomenon. Biosurfactants have enormous applications in industrial effluent bioremediation strategies. Besides this, biosurfactants have been found to be of extensive therapeutic values, like antimicrobials, antiviral, antifungal, and anticancer agents [48]. These wonder molecules can also act as excellent system for drug delivery [59]. Moreover, the inductive effect of toxic *o*-cresol in biosurfactant production is observed in our study. Thus, besides playing a significant role in environmental cleanup, the valuable biosurfactant production can be economically beneficial, thus fitting to the concept of "Waste to Wealth".

Acknowledgments

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