



A Rapid and Simple Method for Screening Microorganisms with a Potential for Catechol Biodegradation

Natalia Filipowicz¹ · Hubert Cieśliński¹

Received: 15 April 2019 / Revised: 30 October 2019 / Accepted: 9 December 2019 / Published online: 19 December 2019
© University of Tehran 2019

Abstract

Catechol is a highly toxic compound that is also a key intermediate in biodegradation pathways of various aromatic compounds. In this paper, a new screening method for isolation of microorganisms with the potential for catechol biodegradation is reported. The method described is based on the ability of catechol to form a color complex with Fe^{3+} ions. For this purpose, basal medium that is widely used for classification of microorganisms by carbon requirements was used. Specifically, minimal Bushnell Haas medium plates supplemented with catechol at 500 mg l^{-1} concentration were used for both yeast and bacteria cultivation. After appearance of microorganism colonies on the growth medium, each plate was flooded with 7.0 ml of 5% (w/v) of FeCl_3 and incubated for a period of 5 min at room temperature. Flooding with this solution led to the formation of a green color from Fe^{3+} -catechol complexes in the growth medium and an appearance of clear zones around colonies of microorganisms that utilize catechol as a sole source of carbon. Based on this, the presented method could be useful as a screening step for microorganisms with a potential for catechol biodegradation.

Article Highlights

- A new screening method for isolation of microorganisms with the potential for catechol biodegradation has been proposed.
- The presented method is based on the ability of catechol to form a color complex of catechol and Fe^{3+} ions and appearance of clear zone around colonies of microorganisms which utilize catechol.
- Described method is simple, not labor-intensive, does not require a long period of time to achieve unmistakable results.
- This method may be also used as a starting point for screening microorganisms with the potential for other aromatic hydrocarbons degradation, in cases when catechol acts like a key intermediate in their biodegradation pathway.

Keywords Catechol utilizing microorganisms · Screening method · Catechol- Fe^{3+} complex · Clear zone · Petri-dish assay

Introduction

Aromatic compounds are widely distributed in the environment and are the main causes of water and soil pollution. High concentrations of these compounds are found mostly in industrial effluents, such as from oil refineries and coal conversion plants (Aleksieva et al. 2002).

One of the most important hazardous aromatic compounds, based on its toxicity, is catechol. Catechol is an aromatic hydrocarbon with two hydroxyl residues at the first and second carbon positions, known also as pyrocatechol or 1,2-dihydroxybenzene. Because of the fact that it is

✉ Natalia Filipowicz
filipowicz.natalia@wp.pl
Hubert Cieśliński
hcieslin@pg.edu.pl

¹ Department of Molecular Biotechnology and Microbiology, Gdańsk University of Technology, Narutowicza 11/12, 80-233 Gdańsk, Poland

widely used (IARC 1999; Schweigert et al. 2001), catechol-contaminated wastewaters are generated by chemical, dye, and photographic and cosmetic industries. Besides industry, catechol can be used to detect and determine concentration of many metal ions in analytical laboratories (Lee 2016). In comparison with other aromatic hydrocarbons, especially phenol, catechol biodegradation has not been well investigated. This is likely due to the fact that catechol is considered more toxic than phenol (Kumar et al. 2005). Only a few studies have focused on the catechol biodegradation process by various microbes including *Pseudomonas* spp. (Kumar et al. 2005; Mrozik et al. 2007; Tewari and Malviya 2002; Zeyaulah et al. 2009), *Achromobacter xylosoxidans* (Bramhachari et al. 2016), *Aspergillus awamorii* (Stanchev et al. 2008), *Candida parapsilosis* (Rigo et al. 2010), *Sphingomonas subarctica*, *Ralstonia pickettii*, and *Comamonas testosteroni* (Di Gioia et al. 2002).

Regarding the biodegradation process, it is crucial to search for microorganisms with high potential to utilize toxic aromatic compounds, both monocyclic, e.g., catechol, and polycyclic aromatic hydrocarbons. These microorganisms may be isolated from areas that are potentially contaminated with these compounds. Microorganisms that are able to grow in such harsh conditions may also be able to degrade these toxic compounds in laboratory studies and may further be used to reduce level of toxic compound in soil or groundwater of other polluted sites (Shishir et al. 2019; Farahani et al. 2010).

To the best of our knowledge, a rapid and simple method that could identify microorganisms with the potential of catechol biodegradation is lacking. To date, the isolation of catechol degrading microorganisms is performed using the method dedicated for screening of microorganisms for the ability of aromatic hydrocarbon biodegradation. For this purpose, one of the most commonly used methods is the slant culture method (Middelhoven et al. 1991). Briefly, in this method, microorganisms are inoculated on the slant surface and compounds being tested reach strains through diffusion or gas phase. Another widely applied method involves isolation of microorganisms directly from soil or water samples with a use of solidified growth medium supplemented with selected aromatic compounds that are the sole carbon source for microorganisms in growth medium (Wang et al. 2012). In another common method, the environmental sample is mixed with liquid medium supplemented with a selected aromatic compound (enrichment step), and then, isolation of microorganisms degrading this compound is conducted using the procedure described above (Kafilzadeh et al. 2010).

Catechol is able to form stable, strong, and reversible complexes with metal ions, like Fe^{3+} , Cu^{2+} , Ca^{2+} , Mn^{2+} , Mn^{3+} , Zn^{2+} , Ti^{3+} , and Ti^{2+} . Moreover, catechol is not only able to form these complexes with soluble metal ions but

also with metal oxide surfaces (Meng et al. 2015). In case of interactions with Fe^{3+} , catechol gives green color with ferric salts, for example, FeCl_3 . In the pH range 2–4, mixing Fe^{3+} and catechol results in a formation of an intense green color (Al-Abadleh 2015; Tewari and Malviya 2002).

The ability of catechol to form complexes with Fe^{3+} is also observed in nature. Catechol- Fe^{3+} complexes were detected in the pigment of mushroom, *Cortinarius violaceus*. Interestingly, the color of the pigment changes in a pH-dependent fashion. This occurs due to the change of a cation:ligand ratio. An increase in the pH leads to the typical pigment color changes from green (pH 3, 1:1) to blue-violet (pH 6, 1:2) and finally bordeaux (pH 9, 1:3) (von Nussbaum et al. 1998).

Based on these properties of catechol, the purpose of this study was to develop and test the suitability of a simple method to detect catechol biodegradation by microorganisms, based on formation of a colored complex between catechol and Fe^{3+} in solid growth medium, and an appearance of a clear halo around colonies of microorganisms which utilize catechol as a sole source of carbon.

Materials and Methods

All experiments were carried on Bushnell Haas (BH) plates. It is a minimal medium recommended for microbial examination of fuels and microbial biodegradation of hydrocarbons that are added to this medium as a sole source of carbon (Hemalatha and Veeramanikandan 2011; Jayanthi and Hemashenpagam 2015).

Bushnell Haas plates contained 0.2 g of magnesium sulfate, 0.02 g of calcium chloride, 1.0 g of monopotassium phosphate, 1.0 g of dipotassium phosphate, 1.0 g of ammonium nitrate, 0.05 g of ferric chloride, and 20 g of bacteriological agar per liter.

Method Development

To determine the optimal conditions for the test, the following experiments were performed. Each BH plate was supplemented with catechol at final concentrations of 500 mg l^{-1} , 750 mg l^{-1} , 1000 mg l^{-1} , 1500 mg l^{-1} , or 2000 mg l^{-1} . A stock solution of catechol (10 g l^{-1}) was sterilized by filtration. Each plate, containing a different catechol concentration, was flooded with a FeCl_3 solution. Three FeCl_3 solutions of 1%, 5% and 10% were applied. Each solution was used in a volume of 2.5 ml, 5.0 ml, or 7.0 ml. Plates were incubated with the FeCl_3 solutions for 1, 2, 3, or 5 min. After the specified period of incubation, the FeCl_3 solutions were decanted from plates.

Method Optimization

The results indicated that the optimal conditions for the test were BH plates supplemented with catechol at 500 mg l⁻¹ concentration, plates flooding with 7.0 ml of the 5% FeCl₃ solution and incubation for 5 min. These parameters were selected due to the intensity of the resulting green complex of catechol-Fe³⁺ ions and the fact that catechol is highly toxic, even more than phenol or resorcinol. Moreover, the green color tends to darken during the incubation time to dark green or even a black color. Hence, after 5 min, FeCl₃ solution was removed from plates.

Because of that, for further experiments, a concentration of catechol equal to 500 mg l⁻¹ was selected as a concentration which should not be lethal for microorganisms with an effective biodegradation potential (Kumar et al. 2005).

Method Usability Determination

To determine the usability of the test, microorganisms from soil samples from two locations were isolated. One of them was an area of oil refinery Lotos Gdańsk (Pomeranian Voivodeship, Poland, 54° 20' 43" N 18° 43' 43" E) that may be contaminated with petroleum-derived waste. The second location was peat land located near Bydgoszcz (Kuyavian-Pomeranian Voivodeship, Poland, 53° 03' 32" N 17° 54' 17" E). Peat lands are naturally rich in polyphenols and phenol derivatives which are products of lignocellulosic biomass decomposition (Thormann et al. 2007).

Sterile 50 ml conical centrifuge tubes (Sarstedt, AG & Co., Germany) were used to collect samples from depth about 5 cm. All samples were transported immediately after collection to the laboratory, and kept on ice to maintain the temperature around 4–8 °C.

For microorganism isolation, 1 g of each soil sample was transferred to a sterile 50 ml conical centrifuge tube and mix vigorously with 9 ml sterile 0.9% saline solution for 10 s (Kim et al. 2008; Soudi and Kolahchi 2011; Wang et al. 2012). After soil sedimentation, 100 µl of each sample was spread with a sterile rod on BH plates supplemented with catechol at 500 mg l⁻¹. For quality assurance, each sample was tested three times. All plates were incubated at 18 °C for 2–3 days. After this period, the growth of microorganisms could be seen as colonies on the surface of the plates. Next, plates were flooded with 7.0 ml of 5% FeCl₃ and incubated for 5 min. During this period of time, the growth medium changed from light amber to dark green. After that, the FeCl₃ solution was decanted and plates were analyzed for the presence of microorganism colonies with the ability to degrade catechol, characterized by the presence of a clear halo around each of them.

To ensure quality of the detection process, in cases where colonies grew close to each other with no possibility of

precisely determining which colony caused the halo appearance, the presented procedure was modified. After soil sedimentation, 100 µl of each sample was used for preparation of serial dilutions in sterile 0.9% saline solution. Then, 100 µl of 10⁻²–10⁻⁵ dilutions were spread with a sterile rod on BH plates supplemented catechol at 500 mg l⁻¹. The remaining steps of the assay were the same as presented above.

Moreover, to obtain reproducible test results, it is important to use freshly poured Petri dishes with BH medium supplemented with catechol. For this purpose, the catechol solution should be prepared and added to BH medium immediately before pouring Petri plates. The FeCl₃ can be prepared occasionally, then stored at room temperature in glass bottles, and used as needed. Both catechol and FeCl₃ solutions need to be prepared with freshly sterile deionized water and then sterilized using filter sterilization (0.2 µm diameter, VWR International Sp. z o.o., Poland).

Results and Discussion

The first indicator demonstrating that isolates from both locations should be able to utilize catechol as the sole source of carbon was their growth on minimal medium BH plates supplemented only with catechol. More importantly, when isolates had an ability to degrade catechol, clear zones appeared around their colonies as a consequence of both catechol consumption and a lack of the possibility of catechol-Fe³⁺ complex formation (Fig. 1). Some colonies of microorganisms isolated from both locations were able to grow on plates supplemented with catechol, but no clear zones around them were observed. In these cases, after the second or third streaking on the test medium, no growth was observed, which resulted from the inability of these isolates to utilize catechol for growth. To confirm the microorganisms' ability of biodegradation of catechol, colonies were streaked once a week (three times), the FeCl₃ test was repeated and the clear zones around colonies were examined.

In summary, the proposed method allows for the rapid and simple identification of microorganisms with the potential for catechol degradation. The appearance of the clear zone around a growing colony, due to a lack of catechol in sites where microorganism utilized that compound as a carbon source, gives an unambiguous visual effect. In comparison to previously described, widely used screening methods aimed at detection of microorganisms with the potential for aromatic hydrocarbons biodegradation, the procedure here stands out as having several advantages. First, the possibility of observing both microorganism colony appearance and reduction of catechol from solid media by this colony (clear halo around a colony), allows the unmistakable selection of microorganism that have the ability to degrade catechol. In the cases of the slant culture method or of plates

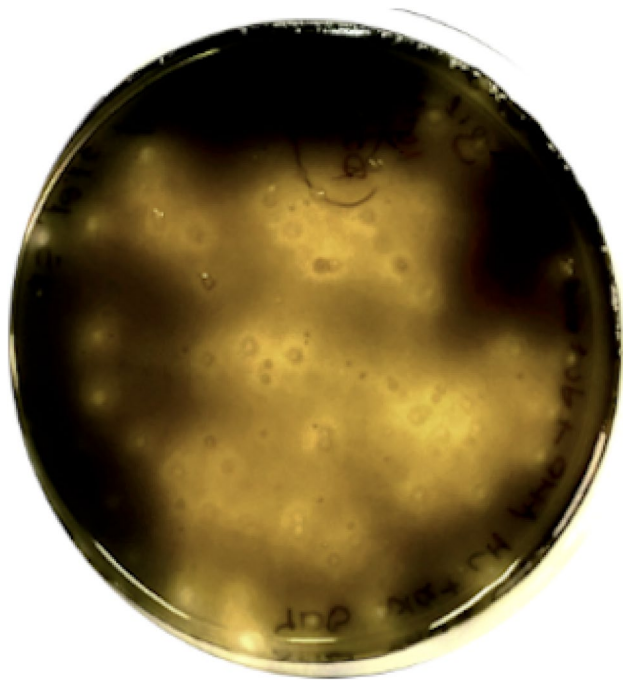


Fig. 1 Growth of isolates from a sample of soil from peat land located near Bydgoszcz, Poland (53° 03' 32" N 17° 54' 17" E) on BH plate supplemented with catechol at 500 mg l⁻¹, after plate was flooded with a 5% FeCl₃ solution. Clear halos indicate the growth of isolates with the ability to utilize catechol as a sole source of carbon

supplemented with appropriate aromatic hydrocarbons, the appearance of colony growth is not necessarily equivalent to compound biodegradation. In both plate and slant methods, colony growth is detected, while a visible effect of biodegradation of certain aromatic compounds is not observed. Moreover, tests based on the growth, or absence of growth, of colonies do not always allow selection of microorganisms exhibiting biodegradation potential. For example, during our previous study, we found that some microorganisms can grow on minimal medium supplemented with the aromatic compound phenol (Filipowicz et al. 2017). Six yeast strains were identified based on their intensive growth on slants supplemented with phenol, but only three of them exhibited intense growth in liquid medium with simultaneous reduction of phenol concentration, which was confirmed by gas chromatography. This leads to the conclusion that growth of isolates on slants or plates supplemented with the analyzed compound is not sufficient for determining that certain microorganisms performed biodegradation, because some isolates may grow using their storage material. Therefore, the slant method needs the next passage of the isolates on the selective medium to reduce false-positive results. Furthermore, the use of the other method based on the mixing of the environmental sample and broth medium supplemented with a selected aromatic hydrocarbon, the sole source of carbon for microorganisms growth (Kafilzadeh et al. 2010;

Wang et al. 2012), there is no possibility of direct selection of specific isolates that are able to biodegrade the selected aromatic compound. These methods are useful mainly to indicate the biodegradation potential of the whole analyzed sample, which contains a mixture of microorganisms. In comparison to those methods, the method proposed here uses chemical properties of catechol to identify microorganisms that are able to use this highly toxic monocyclic aromatic compound as a sole source of carbon. In our opinion, the growth of a microorganism colony on the selective medium using catechol as a sole carbon source, and the visual effect of catechol biodegradation in growth medium, which can be visualized by lack of the presence of dark green color of Fe³⁺-catechol complex, may minimize the possibility of selecting microorganisms that gave the false-positive results during the screening step. Moreover, after the screening for potential catechol degradation by microorganism, all of the selected strains were cultivated in a liquid medium and their ability to degrade catechol was confirmed by gas chromatography (data not shown).

Additionally, compared with methods based on the use of liquid culture supplemented with catechol, the method presented here allows for direct isolation of microorganisms with biodegradation ability from the analyzed environmental sample. In the case of catechol supplemented liquid culture, there is no possibility of direct selection of specific isolates with the ability to biodegrade catechol, and this method is useful only to indicate the potential of the whole sample to carry out this process (Kafilzadeh et al. 2010; Middelhoven et al. 1991; Wang et al. 2012).

From a technical perspective, the proposed method is not labor-intensive and does not require a long period of time to achieve unmistakable results, whereas these issues are associated with the methods already established.

Moreover, this method could also be valuable for detection of microorganisms with potential for degrading other aromatic hydrocarbons, in cases when catechol acts as a key intermediate in their biodegradation pathway, for example phenol. Catechol plays an important role in the biodegradation pathways of various aromatic hydrocarbons. It is a crucial intermediate in the biodegradation pathways of both substituted (mono or in 1,2-orientation) and non-substituted mono and polyaromatic hydrocarbons (Fig. 2) (Bull and Ballou 1981; Nair et al. 2002). Aerobic biodegradation of aromatic compounds involves their conversion into dihydroxylated intermediates (catechol or its substituted derivatives). For example, in the case of phenol, this step involves phenol hydroxylase (monooxygenase), which initiates the oxygenation whereby the aromatic ring is monohydroxylated by a phenol hydroxylase at a position *ortho* to the phenol hydroxyl group to form catechol. In the next step, catechol as a major ring cleavage intermediate is degraded by catechol-1,2-dioxygenase or catechol-2,3-dioxygenase via the

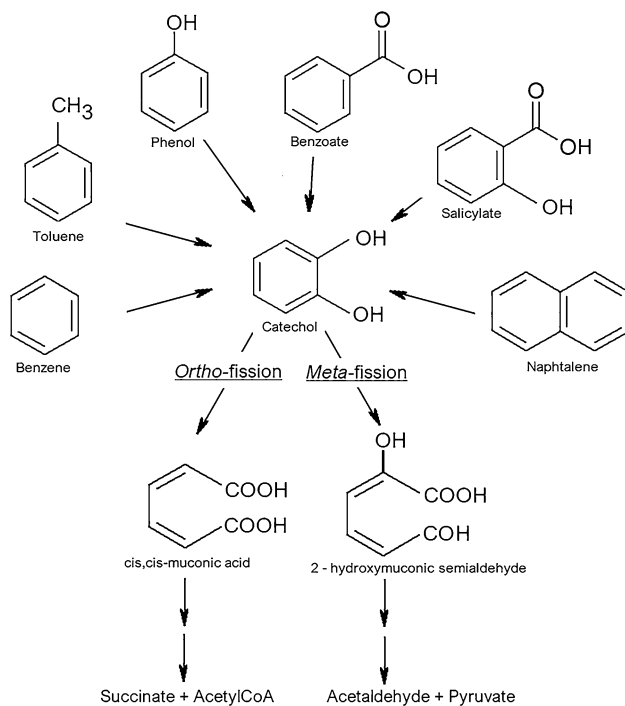


Fig. 2 Examples of aromatic compounds, e.g., benzene, toluene, naphthalene, benzoate, salicylate, and phenol, biodegraded by microorganisms with catechol as the main intermediate of *ortho*- and *meta*-biodegradation pathways

ortho- or *meta*-pathways, respectively (Fig. 2). The final products of both pathways are molecules that can enter the tricarboxylic acid cycle (Tsai et al. 2005; Zeyaulah et al. 2009). For this purpose, three psychrotolerant yeast strains, *Candida subhashii* A01₁, *Candida oregonensis* B02₁, and *Schizoblastosporion starkeyi-henricii* L01₂, which are able to degrade phenol in liquid cultures at a wide spectrum of

concentration (500 mg l⁻¹–1000 mg l⁻¹) (Filipowicz et al. 2017), were tested using the procedure described above. In previous experiments, these three strains were selected according to Middelhoven method (1991). For all three psychrotolerant yeast strains with the confirmed ability to utilize of phenol, the proposed method demonstrated their potential for catechol degradation. Each strain was able to grow on BH plates supplemented with catechol at 500 mg l⁻¹ concentration, and after plates were flooded and incubated with a FeCl₃ solution, the clear zones appeared (Fig. 3). On the other hand, due to the fact that catechol is a common intermediate in biodegradation pathways of many environmental pollutants such as phenol, toluene and benzene (Fig. 2), this method may be also used as a starting point in screening for microorganisms with the biodegradation potential for the above-mentioned and other aromatic compounds.

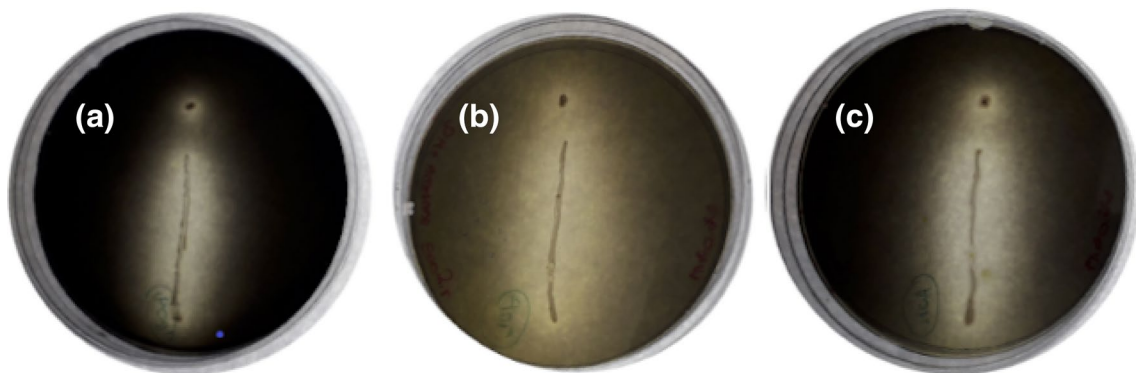


Fig. 3 Growth of phenol degrading yeast strains (single colony and line streak) isolated from Rucianka peat land **a** *Candida oregonensis* B02₁, **b** *Schizoblastosporion starkeyi-henricii* L01₂, and **c** *Candida subhashii* A01₁ on BH plates supplemented with catechol

at 500 mg l⁻¹, after plate was flooded with 5% FeCl₃ solution. The appearance of clear halos around growing yeast strains indicates that they also utilize catechol (intermediate of phenol biodegradation) as a sole source carbon

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Compliance with Ethical Standards

Conflict of interest On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent N/A.

References

- Al-Abadleh HA (2015) Review of the bulk and surface chemistry of iron in atmospherically relevant systems containing humic-like substances. *RSC Adv* 5:45785–45811. <https://doi.org/10.1039/C5RA03132J>
- Aleksieva Z, Ivanova D, Godjevargova T, Atanasov B (2002) Degradation of some phenol derivatives by *Trichosporon cutaneum* R57. *Process Biochem* 37:1215–1219. [https://doi.org/10.1016/S0032-9592\(01\)00336-3](https://doi.org/10.1016/S0032-9592(01)00336-3)
- Bramhachari PV, Reddy DRS, Kotresha D (2016) Biodegradation of catechol by free and immobilized cells of *Achromobacter xylosoxidans* strain 15DKVB isolated from paper and pulp industrial effluents. *Biocatal Agric Biotechnol* 7:36–44. <https://doi.org/10.1016/j.cbab.2016.05.003>
- Bull C, Ballou DP (1981) Purification and properties of protocatechuate 3,4-dioxygenase from *Pseudomonas putida*. A new iron to subunit stoichiometry. *J Biol Chem* 256:12673–12680
- Di Gioia D, Barberio C, Spagnesi S, Marchetti L, Fava F (2002) Characterization of four olive-mill-wastewater indigenous bacterial strains capable of aerobically degrading hydroxylated and methoxylated monocyclic aromatic compounds. *Arch Microbiol* 178:208–217. <https://doi.org/10.1007/s00203-002-0445-z>
- Farahani M, Mirbagheri SA, Javid AH, Karbassi AR, Khorasani N, Nouri J (2010) Biodegradation and leaching of polycyclic aromatic hydrocarbons in soil column. *J Food Agric Environ* 8:870–875. <https://doi.org/10.1234/4.2010.1867>
- Filipowicz N, Momotko M, Boczkaj G, Pawlikowski T, Wanarska M, Cieśliński H (2017) Isolation and characterization of phenol-degrading psychrotolerant yeasts. *Water Air Soil Pollut* 228:210. <https://doi.org/10.1007/s11270-017-3391-8>
- Hemalatha S, VeeraManikandan P (2011) Characterization of aromatic hydrocarbon rading bacteria from petroleum contaminated sites. *J Environ Prot* 2:243–254. <https://doi.org/10.4236/jep.2011.23028>
- IARC (1999) Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. IARC monographs on the evaluation of cancerogenic risk to humans. 71:433–435
- Jayanthi R, Hemashenpagam N (2015) Optimization of BH medium for efficient biodegradation of benzene, toluene and xylene by a *Bacillus cereus*. *Int J Curr Microbiol Appl Sci* 4:807–881
- Kafilzadeh F, Farhangdoost M-S, Tahery Y (2010) Isolation and identification of phenol degrading bacteria from Lake Parishan and their growth kinetic assay. *Afr J Biotechnol* 9:6721–6726. <https://doi.org/10.5897/AJB10.665>
- Kim JM, Le NT, Chung BS, Park JH, Bae J-W, Madsen EL, Jeon CO (2008) Influence of soil components on the biodegradation of benzene, toluene, and o-, m-, and p-xylenes by the newly isolated bacterium *Pseudomonas spadix* BD-a59. *AEM* 74:7313–7320
- Kumar A, Kumar S, Kumar S (2005) Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194. *Biochem Eng J* 22:151–159. <https://doi.org/10.1016/j.bej.2004.09.006>
- Lee BP (2016) Mussel adhesive-inspired polymers. In: Bruns N, Kilbinger AFM (eds) *Bio-inspired polymers*. Royal Society of Chemistry, Cambridge, pp 322–353. <https://doi.org/10.1039/9781782626664-00322>
- Meng H, Liu Y, Cencer MM, Lee BP (2015) Adhesives and coatings inspired by mussel adhesive proteins. In: Bianco-Peled H, Davidovich-Pinhas M (eds) *Bioadhesion and biomimetics*. Pan Stanford, Singapore, pp 131–166
- Middelhoven WJ, de Jong IM, de Winter M (1991) *Arxula adenivorans*, a yeast assimilating many nitrogenous and aromatic compounds. *Antonie Van Leeuwenhoek* 59:129–137. <https://doi.org/10.1007/BF00445657>
- Mrozik A, Piotrowska-Seget Z, Labuzek S (2007) FAME profiles in *Pseudomonas vesicularis* during catechol and phenol degradation in the presence of glucose as an additional carbon source. *Pol J Microbiol* 56:157–164
- Nair C, Jayachandran K, Shashidhar S (2002) Biodegradation of phenol. *Afr J Biotechnol* 7:4951–4958. <https://doi.org/10.1046/j.1462-2920.2001.00176.x>
- Rigo M, Alegre RM, Bezerra JRMV et al (2010) Catechol biodegradation kinetics using *Candida parapsilopsis*. *Braz Arch Biol Technol* 53:481–486. <https://doi.org/10.1590/S1516-8913201000200029>
- Schweigert N, Zehnder AJB, Eggen RIL (2001) Chemical properties of catechols and their molecular modes of toxic action in cells, from microorganisms to mammals. Minireview. *Environ Microbiol* 3:81–91. <https://doi.org/10.1046/j.1462-2920.2001.00176.x>
- Shishir TA, Mahbub N, Kamal NE (2019) Review on bioremediation: a tool to resurrect the polluted rivers. *Pollution* 5:555–568. <https://doi.org/10.22059/poll.2019.272339.558>
- Soudi MR, Kolahchi N (2011) Bioremediation potential of phenol degrading bacterium, *Rhodococcus erythropolis* SKO-1. *Prog Biol Sci* 1:31–70. <https://doi.org/10.22059/PBS.2011.22457>
- Stanchev V, Stoilova I, Krastanov A (2008) Biodegradation dynamics of high catechol concentrations by *Aspergillus awamori*. *J Hazard Mater* 154:396–402. <https://doi.org/10.1016/j.jhazmat.2007.10.038>
- Tewari L, Malviya P (2002) Biodegradation of catechol by fluorescent *Pseudomonas* for sustainable environment. *J Sci Ind Res India* 61:70–74
- Thormann MN, Rice AV, Beilman DW (2007) Yeasts in peatlands: a review of richness and roles in peat decomposition. *Wetlands* 27:761–773. [https://doi.org/10.1672/0277-5212\(2007\)27%5b761:YIPARO%5d2.0.CO](https://doi.org/10.1672/0277-5212(2007)27%5b761:YIPARO%5d2.0.CO)
- Tsai S-C, Tsai L-D, Li Y-K (2005) An isolated *Candida albicans* TL3 capable of degrading phenol at large concentration. *Biosci Biotechnol Biochem* 69:2358–2367. <https://doi.org/10.1271/bbb.69.2358>
- von Nussbaum F, Spiteller P, Rütth M, Steglich W, Wanner G, Gambelin B et al (1998) An iron(III)-catechol complex as a mushroom pigment. *Angew Chemie Int Ed* 37:3292–3295. [https://doi.org/10.1002/\(SICI\)1521-3773\(19981217\)37:23%3c3292:AID-ANIE3292%3e3.0.CO;2-N](https://doi.org/10.1002/(SICI)1521-3773(19981217)37:23%3c3292:AID-ANIE3292%3e3.0.CO;2-N)
- Wang J, Ma X, Liu S, Sun P, Fan P, Xia C (2012) Biodegradation of phenol and 4-chlorophenol by *Candida tropicalis* W1. *Proc Environ Sci* 16:299–303. <https://doi.org/10.1016/j.proenv.2012.10.042>
- Zeyaulah M, Ahmad R, Naseem A, Islam B, Hasan HMI, Abdelkaf AS et al (2009) Catechol biodegradation by *Pseudomonas* strain: a critical analysis. *Int J Chem Sci* 7:2211–2221