

Biodegradation of endocrine disruptor Bisphenol A by indigenous microbial consortium of waste water: a case study

Abstract:

Bisphenol A (BPA) or 2,2-bis-(4-hydroxyphenyl) propane) has been the most havoc-wrecking polycarbonate pollutant released majorly from plastic and resin manufacturing industries into the municipal wastes. As per USEPA, if it contaminates open water bodies and enters our food chain, it can act as an endocrine disruptor to aquatic creatures and a potential carcinogen to human. Hence, removal of this compound is necessary from the environment. Conventional chemical/physical/mechanical mitigation processes further add on to accumulation of toxic reagents in the environment. Hence, many researchers reported use of few bacterial strains for biodegradation of BPA in sustainable, environment friendly pathways. Strains of *Sphingomonas* sp. MV1, *Sphingomonas bisphenolicum* A01, *Sphingobium* sp. BiD 32, *Citrobacter freundii*, *Pseudomonas* sp. have been reported to degrade 99.87% – 100% BPA within 72-110 hours at rates 1.61-2.2 µg/L/h by using enzyme coenzymes tandem pathways. Laccase and Oxidase enzymes with coenzymes NADH, NAD⁺, NADPH, NADP⁺ performs zero/first order oxidative degeneration reaction of BPA. Reported HPLC, GC-MS analysis showed formation of end products oxalic acid, 1,2,4-trimethylbenzene and 2,9-dimethyldecane which proved to be non-toxic by algal toxicity testing. This information can further help future researchers to genetically engineer the established strains for faster, cost-effective mitigation of BPA, in a green-technological mechanism.

Keywords: endocrine disruptor chemical, Bisphenol A, biodegradation, microbial consortium

1. Introduction:

The most prevalent environmental contaminants are polycyclic aromatic hydrocarbons, or PAHs. Numerous individuals worldwide are exposed to these substances, which are widely dispersed throughout a variety of habitats, including water supplies. A man-made PAH with two phenol rings, bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl) propane; CAS registry no. 80-05-7) is used extensively in the manufacturing of synthetic polymers, especially epoxy resins and polycarbonate plastics (Atacag et al., 2015). This substance has been identified as one of the endocrine-disrupting compounds (EDCs) that can impact human and other organisms' reproductive because of its androgenic or oestrogenic action, despite its significant industrial uses (Wang et al, 2017). Additionally, a number of studies have demonstrated that BPA has mutagenic, carcinogenic, immunotoxic, and embryotoxic effects that pose a major risk to both human and environmental health (Alexander et al., 1988). These days, the increased manufacturing of BPA is a result of the extensive global demand of plastic products. Thus, during production, significant levels of BPA may be discharged into the environment, particularly in industrial and municipal wastewaters.

Transportation or consumption procedures. It is therefore imperative to provide an effective, environmentally acceptable method for removing it from exposed natural habitats. Based on our earlier research and the findings of other investigations, bioremediation employing bacteria that break down BPA has been shown to be an efficient method of getting rid of this substance. Numerous BPA-degrading bacteria have been identified thus far from soil, sediment, water, and petrochemical wastes, and the majority of them contain BPA-biodegradation routes. Lobos and colleagues (19) and Spivack and colleagues (30) were the first to give an explanation of the two main and secondary mechanisms that *Sphingomonas* sp. strain MV1 uses to biodegrade BPA. The primary mechanism that has been suggested generates 4-hydroxyacetophenone and 4-hydroxybenzoic acid.

1.1 As metabolic intermediary compounds, a novel indigenous *Pseudomonas pseudoalcaligenes* bioremediates a salty petrochemical wastewater containing bisphenol A, while the minor one yields 2,2-bis(4-hydroxyphenyl)-1-propanol and 2,3-bis(4-hydroxyphenyl)1,2-propanediol as primary metabolites. Later, in addition to confirming these pathways in other bacterial strains, distinct metabolic pathways were also identified in particular bacterial strains, such as *Pseudomonas aeruginosa* PAb1 isolated from thermal paper industry effluent by Vijayalakshmi et al. in 2018 and *Bacillus pumilus*

strains BP-2CK, BP-21DK, and BP-22DK isolated from kimchi by Yamanaka et al. in 2007. BPA can be broken down by bacteria that break down sphingosine, including *Bacillus*, *Ochrobactrum*, and *Picocystis* sp. However, microbes have a hard time breaking down BPA due to its benzene ring structure. Thus, additional carbon or nitrogen sources, like yeast or glucose, are typically required for screening individual species of bacteria to enhance their BPA breakdown. Nevertheless, a suitable strain of bacterial resources for the breakdown of BPA is still absent because this process will make degradation more complex and incur additional operating and maintenance expenses. BPA was thus the sole carbon source in this experiment to identify microorganisms capable of effectively breaking down BPA and investigate how they degraded in water.

1.2 Through enzymatic processes controlled by functioning genes, microorganisms can frequently break down contaminants. Microorganisms will use the metabolic regulatory mechanisms to withstand BPA stress during the microbial breakdown of BPA. They are deteriorating it. Researchers can better grasp the key elements in the degradation process by identifying the mechanism of BPA breakdown in microbial cells and screening the most significant genes engaged in this process. Though more research and analysis are required, some progress has been made in understanding the molecular mechanism underlying the biodegradation of BPA. Some studies have looked at how specific elements, such temperature, a solution's acidity, the condition of the bacteria, or bacterial metabolites, affect degradation.

1.3 Despite extensive research on the BPA degradation pathways, little is known about the essential genes and metabolic processes involved in BPA degradation. Consequently, the analysis of a microbial genome is a crucial phase that can be applied to further our understanding of the genome, particularly with regard to the defence and degradation mechanisms of bacteria associated with BPA. This will encourage the development of accurate bioremediation techniques for contaminated areas. Since BPA is one of the most prevalent endocrine disruptors in the environment, it is essential to screen for bacteria that break down BPA and investigate their genome.

1.4 In order to ascertain strain P1's degradation capability under the impact of various environmental variables, qPCR was used to confirm the expression of genes encoded by enzymes involved in BPA degradation. The defence mechanism of bacteria against harmful contaminants was examined and explained based on functional annotation. The goal of this research is to provide a theoretical framework for microbial remediation of

BPA-polluted settings, enhance the BPA degradation and resistance mechanism of strains, and further enrich the bank of bacteria that break down BPA.

1.5 Several BPA-degrading bacteria have been reported but most of them are only able to degrade low amounts of high BPA concentrations within several days in lab conditions and thereby cannot be used for practical removing of this compound. Hence, in the present study, isolation and characterization of a novel indigenous BPA-degrading bacterium for elimination of BPA from salty wastewater was considered. Potential of isolated bacterium for BPA removal from petrochemical wastewater was investigated in lab scale. The possible metabolic pathway for BPA biodegradation by isolated bacterium were also proposed by identification of the metabolic intermediary compounds using high performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC/MS) analysis.

2. CASE STUDY:

Vijayalakshmi et al. in 2018, established *Escherichia coli* (DH5a) as a strain for gene cloning (Novagen, Germany). BPA, 4 hydroxybenzaldehyde (4-HBAL), 4-hydroxybenzoic acid (4-HBA) and 4-hydroxyacetophenone (4-HAP) with purity of 99% were purchased from Alfa Aesar (Spain). All chemicals, enzymes, plasmids and kits were purchased from specific manufacturers. Solvents for HPLC were of HPLC grade. They used the following Media and Growth Conditions: Basal salt medium (BSM, containing 1.0 g K₂HPO₄, 1.0 g (NH₄)₂SO₄, 0.2 g MgSO₄·7H₂O, 0.01 g FeCl₃, 0.05 g NaCl and 0.05 g CaCl₂ per liter, pH 7) and Luria-Bertani (LB) medium (10 g Peptone, 10 g Na Cl, 5 g Bacto Yeast extract per liter, pH 7) were used for isolation and cultivation of BPA and phenol utilizing bacteria. BPA was added to BSM and LB medium through 2 methods. In some experiments, BPA as a sole carbon source was added to the above-mentioned media before autoclaving at an initial concentration of 300 mg/L of LB-BPA, unless other concentrations stated. In the other experiments, BPA solution (1 g/L) was prepared by dissolving 100 mg of BPA in 5 mL of pure ethanol (99%) and adding distilled water up to 100 mL and resulting BPA solution was added to BSM (BSMBE); at initial concentrations of 300 mg/L and 1% (v/v), for BPA and ethanol respectively. Growth and BPA-degradation activity of the selected bacterial isolates in the original petrochemical wastewater were also confirmed in 250 mL Erlenmeyer flasks containing Khuzestan petrochemical wastewater (PWW) and supplemented with concentrated solutions of BSM

(PWW-BSM) and 200 mg/L BPA. After incubation of cultures with rotary shaking in dark (200 rpm at 30°C), growth was monitored based on absorbance at 600 nm (OD600) spectrophotometrically (Beckman, USA) during different cultivation times. Media having 300 mg/L BPA and 1.5% (w/v) pure agar were used for colony purifying and growth of the individual isolates of the bacterial consortium (from 2 to 4 days of incubation at 30°C). For isolation of BPA-resistant bacteria and its use in degradation of the same, the authors performed primary screening. In primary screening experiments, 1 mL of each petrochemical wastewater samples were inoculated directly into 50 mL of BSMBE (100) containing 20 g/L NaCl. The resultant cultures were incubated at 200 rpm and 30°C for 7 days. When the turbidity was appeared, 1 mL of the grown culture media was transferred in a stepwise manner into 50 mL fresh BSMB, containing 40 g/L NaCl for secondary screening. Finally, the grown bacterial cells in BSMB containing 40 g/L NaCl were cultivated on BSMB agar plates. The morphologically distinct bacterial isolates were purified on the BSMB plates by repeated streak plate method and stored in 30% (v/v) glycerol and 1% (v/v) tryptone solution at -70°C. Certain effects of temperature and pH were also checked: 5 mL of LB medium was inoculated with a colony of selected isolate from agar plate. The culture was incubated at 37 °C and 200 rpm until OD600 of 0.6. Thereafter, the resultant pre-culture was inoculated into 50 mL of BSMB containing 40 g/L NaCl at final OD600 of 0.2. Cultures were incubated under shaking (200 rpm) at 25, 30, 35 and 40 °C for 48 h. At 12 h intervals, the OD600 of each culture was determined spectrophotometrically (Beckman, USA). The effect of pH on the growth rates of the selected isolate was determined by cultivation of pre-culture in BSMB containing 40 g/L NaCl media with pH 5, 6, 6.5, 7, 7.5, 8 and 9 under the same above-mentioned conditions. During the growth of the cultures, OD600 were determined periodically as described previously. The authors also checked Chemical Oxygen Demand of degrading bacteria: The cells from log-phase culture (18 h at 37 °C) of selected isolate in LB medium were harvested by centrifugation (5000 rpm, 20 min). The pellet was re-suspended in BSM medium and washed twice. Then, appropriate amount of the resulting suspension was inoculated in 50 mL BSMB containing 40 g/L NaCl to obtain an initial absorbance of 0.2 at 600 nm. The culture was grown in an incubator shaker at 30 °C and 200 rpm for 48 h. Samples were collected from the culture at time points of 6, 12, 18, 24, 30, 36, 42 and 48 h and centrifuged at 13 000 rpm for 5 min. The supernatant was used for estimation of COD, whose values indicated the mean value of the two independent determinations repeated each time in duplicate. Determination of BPA Degradation: Selected isolate was pre-

cultured in LB medium and grown aerobically under shaking (200 rpm) for 18 h at 37 °C. The cells were centrifugally separated (5000 rpm, 20 min) and washed twice with 5 mL of fresh culture medium. The cells inoculated into BSMB containing 50 mg/L PWW (petrochemical wastewater), PWW-BSM media at an OD600 of 0.2 and incubated under shaking (200 rpm) at 30 °C for 48 h. After incubation, samples (1 mL) were collected from the cultures at certain time points and centrifuged at 13,000 rpm for 5 min. The resultant supernatants were filtrated through a 0.2 μ membrane filter (Millipore, USA). The amount of phenol and BPA in the filtrates were determined by a high-performance liquid chromatography (HPLC) and reverse phase C18 column (4.6 $\times$ 250 mm, 5 mm Zorbax RX-C18). The samples were eluted with a linear gradient (10-90% acetonitrile-water) at 1 mL/min for 40 min. The injection volume was 25 μ L and the absorbance was monitored at 280 nm. Identification of metabolites from BPA decomposition with gas chromatography-mass spectrometry (GC/MS) and HPLC was also carried out by the authors as follows. Three compounds of 4-HAP, 4-HBAL, and 4-HBA acid were previously reported as BPA degradation metabolites of *Sphingomonas* sp. strain MV1, *Pseudomonas alkylphenolica* and other bacterial strains in the KEGG database. For identification of metabolic intermediary compounds of BPA-biodegradation pathway in the selected isolate, the standard solutions of three above mentioned compounds (200 mg/L) and BPA (300 mg/L) were prepared in the BSM medium and analyzed by HPLC. The metabolites derived from the biodegradation of BPA by selected isolate were identified based on comparison and matching of the peak retention time belonged to known (standard) and unknown compounds. Process of BPA Removal: Pre-culture for fermentation was prepared by inoculating 200 mL LB medium with a single colony of selected isolate. Flasks were incubated 18 h at 37°C aerobically under shaking at 200 rpm. Batch fermentation was carried out in a 2 L fermenter with 1.2 L working volume (Biolog 3000; New Brunswick Scientific Co., New Jersey, USA). The fermenter was equipped with a built-in controller for pH, temperature, agitation, dissolved oxygen (DO), and peristaltic pumps for base and acid additions. Pre-culture was centrifuged (5000 rpm, 20 min) and collected cells washed twice with fresh BSM medium. The washed cells inoculated into PWW, PWW-BSM at 5% (v/v) in separate batch tests. After inoculation, the temperature and pH of fermenters were automatically maintained at 35°C and 7, respectively. The DO was maintained automatically at 10% by controlling the agitation speed up to 500 rpm. Sampling was carried out at certain time points. Phenol and BPA concentration of samples was determined using HPLC analysis at the same condition mentioned.

3. OUTCOME OF CASE-STUDY:

Each collected sample was inoculated into liquid BSMBE (100) containing 20 g/L NaCl and cultivated at 30°C for 7 days. Considerable turbidity ($OD_{600} = 0.6 - 1.1$) was observed in 4 samples during 1 to 3 days of incubation. For obtaining bacteria with higher BPA-degrading activity in salty conditions, four grown samples were cultured in BSMB containing 200 and 300 mg/L BPA as sole carbon source and 40 g/L NaCl using stepwise enrichment manner. Only one BSMB culture showed turbidity after 24 h; suggesting the existence of BPA-degrading and NaCl-tolerant bacteria in that sample. Colony purification was performed by spreading of the grown liquid culture on BSMB agar plate. All colonies on the solid medium were derived from one bacterial strain on basis of the colony morphology. Consequently, one isolate was selected for further experiments and designated as YKJ isolate. The YKJ isolate was a Gram-negative, catalase and oxidase-positive bacilliform bacterium. Colonies of this isolate on LB agar plates were milk-white (1-2 mm size), non-transparent, circular with convex appearance, and smooth margin. Antibiotics resistance evaluation on LB agar plates containing different antibiotics and antibiogram test, showed sensitivity of YKJ isolate to rifampicin (100 µg/L), kanamycin (50 µg/L), and tetracycline (25 µg/L) and its resistance to ampicillin (50 µg/L) and chloramphenicol (34 µg/L) as previously realized genetically. Growth condition determination: The growth parameters including suitable temperature and pH were determined for YKJ isolate in the presence of BPA as sole carbon and energy sources in salty condition. The results showed that the isolate was able to grow in liquid BSMB containing 40 g/L NaCl at 25, 30, 35 and 40 °C. In addition, measuring OD_{600} of collected samples within 12, 24, 36 and 48 h of cultivation demonstrated the higher growth rate of YKJ isolate at 30°C compared to other temperatures. Therefore, temperature of 30°C was applied as one of the growth parameters in further experiments (Fig. 1).

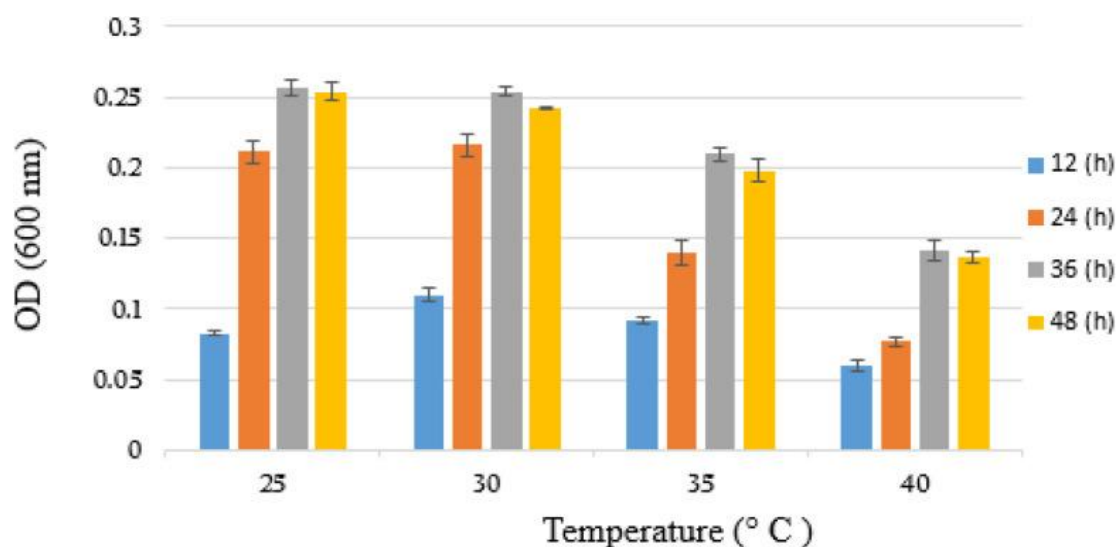


Figure 1: Effect of temperature on growth of BPA-degrading bacteria at OD600

Subsequently, the growth rate (OD600) of YKJ isolate was evaluated in liquid BSMB containing 40 g/L NaCl at pH of 5-9 and temperature of 30°C. OD600 of cultures after 12, 24, 36 and 48 h of incubation showed that YKJ isolate was able to grow at all tested pHs except 5. However, the best growth of this isolate was at pH of 6.5, 7 and 7.5. The growth pattern in different pHs indicated that growth of the YKJ isolate was not limited to a specific pH (Fig. 2). Moreover, the potential of YKJ isolate for growth in high concentrations of NaCl (up to 40 g/L) as well as different pHs (6-9) and temperatures (25-40 °C) can presumably illustrate the ability of the isolate to grow in the conditions existing in the petrochemical wastewater.

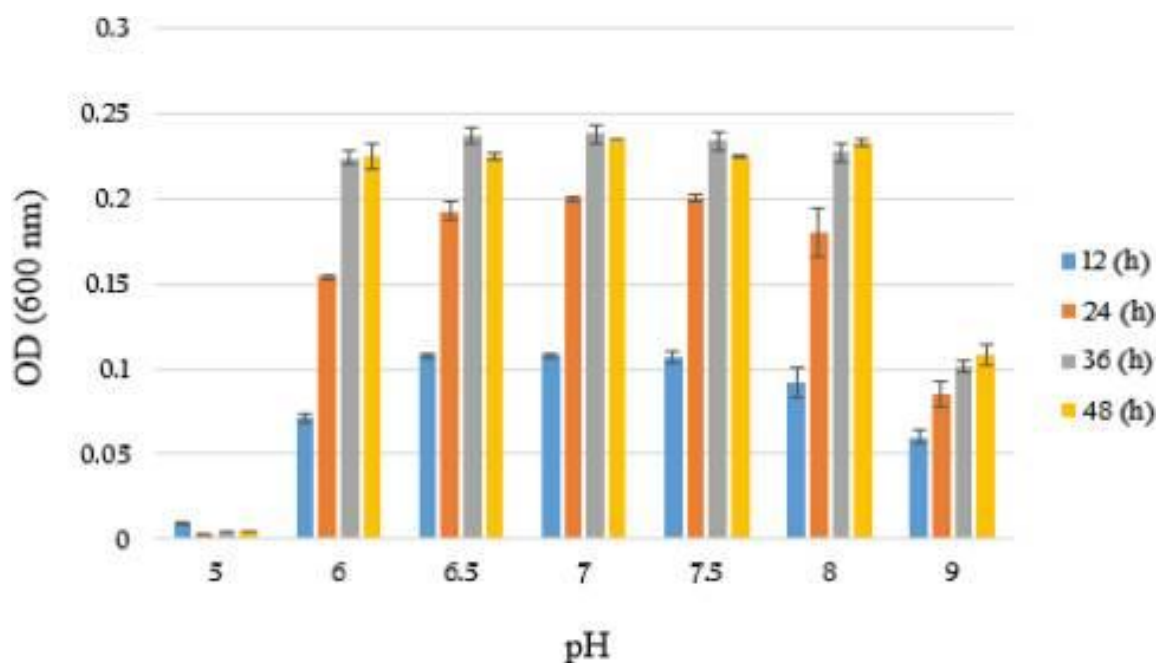


Figure 2: Effect of pH on growth of BPA-degrading bacteria as observed at OD600

Chemical oxygen demand of degrading bacteria:

BPA-degradation activity of YKJ isolate was determined by measuring the COD of the isolate culture in BSMB containing 40 g/L NaCl within 6, 12, 18, 24, 30, 36, 42 and 48 h of cultivation. According to the results, the COD of above-mentioned culture reduced from the initial value of 655.2 mg/L to 109.2 mg/L (about 83% decrease) after 36 hours and remained almost constant up to 48 hours (Fig. 3). Also, growth monitoring of the culture at the same time points showed close correlation between growth rate of YKJ isolate and COD reduction in BSMB containing 40 g L⁻¹ NaCl (Fig. 3). Reducing COD of BSMB culture and increasing the growth of YKJ isolate could indicate the ability of this isolate for utilization of BPA as sole carbon and energy sources

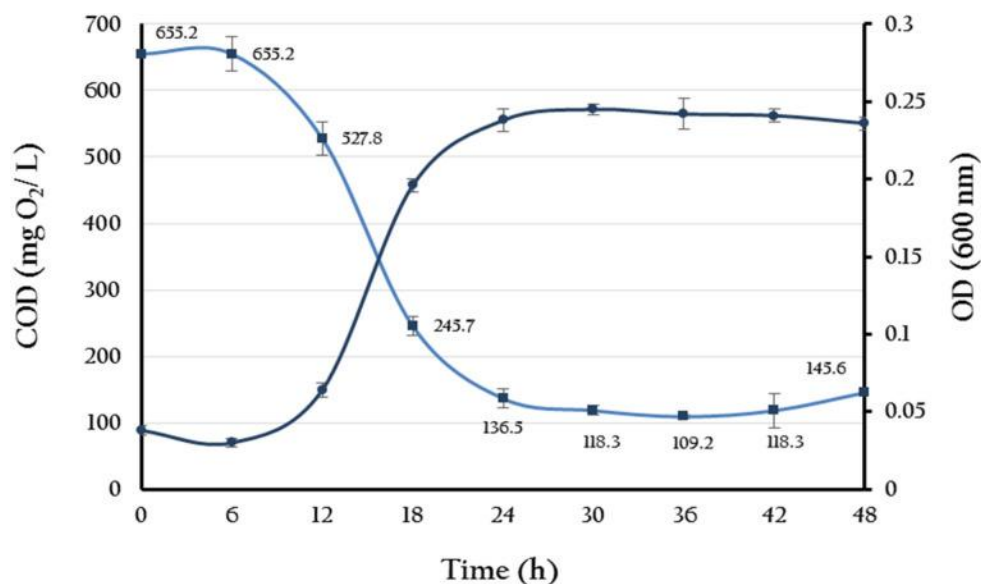


Figure 3: Inter-relation of time, COD, biomass growth of BPA-degrading bacteria

4. PLAUSIBLE MECHANISM OF BPA-DEGRADATION BY ISOLATES

BPA-degradation activity of *P. pseudoalcaligenes* strain YKJ was also confirmed in BSMB containing 40 g/L NaCl by HPLC analysis. According to the chromatogram of the HPLC, the retention time of BPA was 23.772 min (Fig. 4). The calibration curve equation for detection of the BPA concentration was as follows: peak area = 21.12 CBPA - 9.742 ($R^2 = 0.999$), where CBPA was the BPA concentration (within the range of 1 - 300.0 mg/L). The results demonstrated that BPA at 300 mg/L was reduced to 243.7, 97.57, 11.14 and 0 mg/L by *P. pseudoalcaligenes* strain YKJ within 12, 18, 24, and 36 h, respectively. This strain was able to degrade high levels (288.86 mg/L) of BPA within 24 h and utilize 100% (300 mg/L) of BPA without detectable new peaks in HPLC analysis within 48 h. Therefore, *P. pseudoalcaligenes* strain YKJ can utilize BPA as sole carbon source to produce CO₂, H₂O and all cell components.

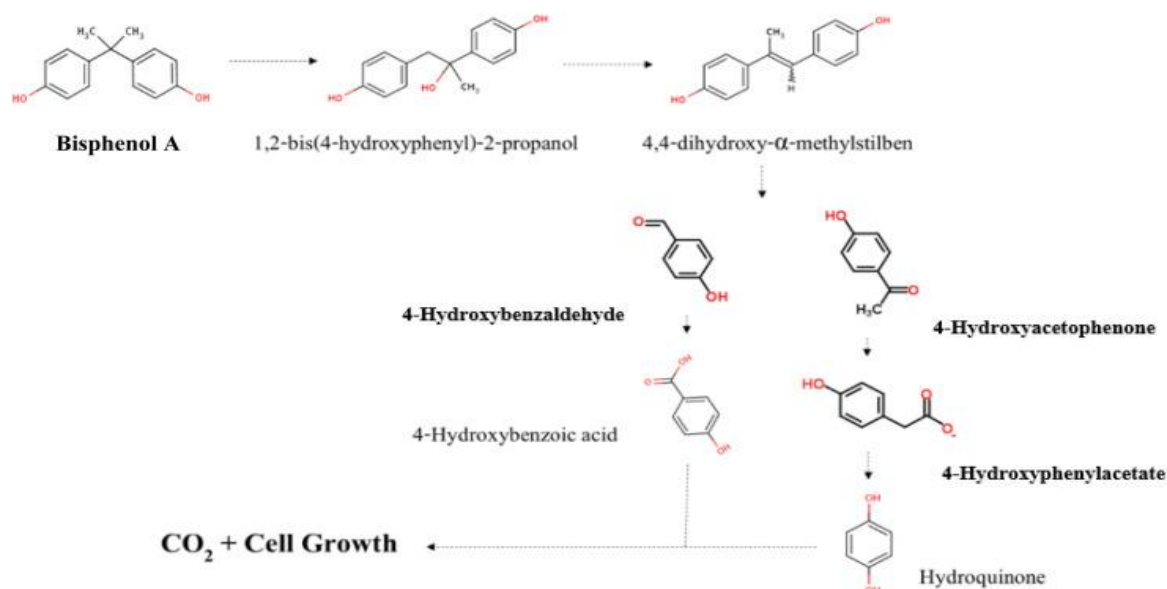


Figure 4: Plausible mechanism of BPA degradation by BPA-degrading isolates

Subsequently, the growth and phenol, BPA-degradation activity of *P. pseudoalcaligenes* strain YKJ were evaluated in PWW and PWW-BSM which contained 100 mg/L phenol and 300 mg/L BPA on the basis of HPLC analysis. Growth increased to OD₆₀₀ of 0.597 ± 0.009 in PWW and OD₆₀₀ of 0.570 ± 0.005 in PWW-BSM until 24 h and thereafter until 48 h, it did not increase. BPA was decreased to an undetectable level by HPLC in both cultures within 24 h. Thus, similarity of the results in both cultures showed that the BSM mineral salts could not stimulate the growth and phenol, BPA-degradation activity of *P. pseudoalcaligenes* strain YKJ in PWW. In addition, this strain was able to grow up to OD₆₀₀ of 0.5 and degrade and 300 mg/L BPA (100%) in PWW and PWW-BSM within 24 h which were higher than that in the BSMB containing 300 mg/L BPA within 48 h. Therefore, it was probable that other organic compounds existing in the petrochemical wastewater could stimulate the growth and degradation activity of *P. pseudoalcaligenes* strain YKJ. In addition, other living microorganisms existing in petrochemical wastewater might synergistically enhance the growth and BPA degradation activity of this strain.

5. COMPARATIVE STUDY OF BPA-DEGRADATION CAPACITY AMONG BPA-DEGRADING STRAINS AS PER REORTED LITERATURE

As observed in the table below (Table1), we can see the comparison of BPA-degradation capacity of the strain discussed in the present case study vis-à-vis other reported literature.

Table 1: Comparison of BPA-degradation capacity of various strains as reported

Microorganism	BPA concentration mg/L	NaCl concentration g/L	BPA removal (%)	Time (h)	Reported Literature
<i>P. pseudoalcaligenes</i> strain YKJ	300	40	96.28	24	Present case study
<i>Pseudomonas putida</i> strain YC-AE1	200	0	100	20	Eltoukhy <i>et al.</i> (2020)
<i>Sphingobium</i> sp. YC-JY1	100	0	100	12	Jia <i>et al.</i> (2020)
<i>Sphingobium</i> sp. YC-JY1	100	6–10	0 (inhibited)	10	Jia <i>et al.</i> (2020)
<i>Pseudomonas</i> sp. strain KU1	1000	0	78	288	Kamaraj <i>et al.</i> (2014)
<i>Pseudomonas</i> sp. strain KU2	1000	0	81	288	Kamaraj <i>et al.</i> (2014)
<i>Bacillus</i> sp. strain KU3	1000	0	74	288	Kamaraj <i>et al.</i> (2014)
<i>Enterobacter gergoviae</i> strain	200	0	11.55	8	Badiefar <i>et</i>

Microorganism	BPA concentration mg/L	NaCl concentration g/L	BPA removal (%)	Time (h)	Reported Literature
BYK-7					<i>al.</i> (2015)
<i>Bacillus</i> <i>pumilus</i> strains BP-2CK	25	10	100	48	Yamanaka <i>et al.</i> (2007)
<i>Bacillus</i> <i>pumilus</i> strains BP-21DK	25	10	100	48	Yamanaka <i>et al.</i> (2007)
<i>Bacillus</i> <i>pumilus</i> strains BP-22DK	50	10	100	120	Yamanaka <i>et al.</i> (2007)

It is also worth noting that biodegradation of PAHs as BPA depends on environmental factors such as temperature, pH, and salinity. These parameters have important effects on the growth of bacteria and catabolic activity of the enzymes involved in the BPA-biodegradation process. The *P. pseudoalcaligenes* YKJ can grow in a temperature range of 25 - 40°C with optimum growth temperature of 30 °C. Increasing the temperature improves solubility of the BPA, and thus, significantly increases the bioavailability of BPA molecules. In return, higher temperature reduces the metabolic activity of mesophilic aerobic microorganisms which is also seen as reduced growth of this bacterium in BSMB containing 40 g/L NaCl. Optimum growth of YKJ strain at 30°C can be due to the optimum temperature for activity of the enzymes involved in the BPA-biodegradation pathway that is lower than 40°C. pH of the medium also affects microbial activity including enzymatic activity, solubility, and accessibility of nutrients. *P. pseudoalcaligenes*

YKJ grows at pH 6–9 with the best growth in the pH 6.5 - 7.5. The growth pattern indicates that the BPA-biodegradation activity of this strain is not limited to a specific temperature and pH. These results again suggest that antibiotic resistance genes are in bacterial plasmids which can be horizontally transferred between environmental bacteria. Therefore, the strains with the least resistance to antibiotics should be considered for potential applications to minimize environmental risks. *P. pseudoalcaligenes* YKJ is resistant to Chloramphenicol and slightly to Ampicillin (which can be removed through genetic engineering approach) but not to other antibiotics. These characteristics may be suited for the bioremediation purpose.

6. CONCLUSION:

The isolation and identification of a novel BPA-degrading *P. pseudoalcaligenes* strain YKJ. It was able to degrade BPA as the only source of carbon and energy in the basal salt medium containing high concentration of NaCl more rapidly than the other reported bacteria. BPA biodegradation pathways by this strain were proposed based on the analysis of the metabolites. Our results showed that strain YKJ was applicable for treatment of salty petrochemical wastewater containing high concentrations of phenol and BPA. A strain of *Pseudomonas* sp. P1, which is capable of efficiently breaking down BPA, was acquired for this investigation. When the temperature was 30 °C, the pH was 7, the BPA concentration was 30 mg/L, and 3 mL of inoculation was used, the maximum breakdown rate was 96.89%. There are 5636 protein-encoding genes in strain P1's genome. All of the critical genes for BPA biodegradation in strain P1 were screened using comparative genomic analysis, including 138 functional genes that may be engaged in BPA degradation and 72 functional genes involved in the mechanism of BPA stress. Under BPA induction, seven genes were expressed, including laccase, ferredoxin, cytochrome P450, and ferredoxin reductase complex. Strong environmental adaptability is exhibited by strain P1, which can withstand temperatures between 25 and 40 °C, pH values between 5 and 8, and BPA concentrations between 15 and 100 mg/L. During the BPA degradation process, six intermediates were identified, including 4-vinylphenol, which was discovered for the first time. The biodegradation pathway was suggested based on this. The findings demonstrate that strain P1 can be utilised for bioremediation of various BPA-contaminated environments due to its abundance of functional genes and strong environmental adaptability.

334 **CONFLICT OF INTEREST:**

335 The authors declare no conflict of interest with anyone.

336

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