Isolation, indentification and degradation of Biphenol A by *Bacillus* sp. from effluents of thermal paper industry

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Abstract - A bacterial strain with a high degrading efficiency towards bisphenol A (BPA), was isolated from thermal-paper industry effluent, and identified as *Bacillus sp* which was based on the morphological, physiological, and biochemical studies and the identity further confirmed by 16S rRNA analysis. The cells were rod-shaped, gram-negative and motile. Monod Model studies showed that the *Bacillus sp* degrades BPA in basal salt medium (BSM) and nutrient agar. The maximum specific growth rate (μ_{max}) values ($0.538h^{-1}$ and $0.556h^{-1}$), half-saturation coefficient K_s values (23.91 and 25.72), and R^2 values (0.99 and 0.98) were recorded for the bacterial species cultured in BSM medium and nutrient agar.. HPLC chromatograms revealed that 20-m*M* concentrations of BPA gradually decreased within 9 days of incubation, which was reflected in a corresponding reduction in the peak area from 100% (initial concentration) to 11.33% (final concentration). During BPA degradation by the *Bacillus sp*, the metabolic intermediates such as, *p*-hydroxyacetophenone, *p*-hydroxybenzoic acid and hydroquinone, detected were identified by GC-MS. BPA degradation was studies using wide range of temperatures (25-45^o C) and pH conditions (5-11) and optimum degradation was observed at 35^{o}C and at a pH of 8.0. The present study reports on an efficient protocol for degradation of BPA that could be of significance for the bioremediation of BPA effluent polluting the environment.

Index Terms: Thermal-paper industry, Bisphenol A degradation, *Bacillus sp*, basal salt medium, Monod equation

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1. INTRODUCTION

Bisphenol A (BPA) is also called 2, 2-bis (4-hydroxy phenyl propane) and consists of two phenolic rings joined through a bridging carbon or other chemical structure. It is an industrially important compound used in the production of polycarbonates, epoxy resins, thermal paper and other plastics in many chemical manufacturing plants throughout the world [1], [2]. BPA acts as a colour and image stabilizer in the production of thermal paper [3], [4]. Manufacturing facilities and treatment processes generate significant quantities of BPA which are discharged into terrestrial, aquatic and marine environments. The compound is also identified as a strong endocrine disrupting substance which causes adverse effects in humanbeings and wildlife [5], [6], [7], [8], [9], [10]. Collectively, these studies suggested that, it is necessary to purge the BPA from the polluted environment. So far, several systems for BPA removal have been proposed, but microbial degradation is expected to play a major role in the removal of Bisphenol A from these environments [11].

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A number of BPA-degrading Bacteria have been isolated from different environments such as enrichments of sludge from waste treatment plants [10],[12], aquatic environments [6], [7], [10], the soil environment [13], [14], [15], effluents of thermal paper industry [16] and compost leachate [17]. In the present study, microorganisms were isolated from effluents of thermal paper industry and these are known to deteriorate the quality of aquatic environments, especially drinking water sources [4], [18].

Generally, BPA is degraded via major and minor pathways, both involving oxidative skeletal rearrangements and an initial hydroxylation step of the carbon atom in the bisphenol molecule. It has been reported that gram-negative bacteria generally degrade BPA by the major pathway and also suggested a relationship between environmental conditions and BPA biodegradation [6], [9], [10], [17]. Physiological conditions play a major role in the biodegradation of BPA. The relationship between the specific growth rate (μ) of a population of microorganisms and the substrate concentration (S) is a valuable tool in biodegradations studies [19]. Till date, degradation of BPA by microorganisms has been optimized only for laboratory-scale conditions and hence it lacks efficacy at high concentrations of BPA [20]. In the present investigation, a bacterial isolate from the effluent of a

thermal paper industry was studied for its ability to degrade BPA and the possible metabolic pathway involved in degradation was discussed. The degradation activity of BPA by *Bacillus sp* was monitored by HPLC and Monod kinetic models (μ_{max}) as applied to the prediction of microbial growth and degradations.

2. MATERIALS AND METHODS

2.1. Reagents and sample collection

Bisphenol A (BPA) was commercially purchased from Sigma-Aldrich. Effluent samples were collected in sterile screw-capped bottles from a flow region of 5-10 metres downstream of the effluent discharge point, transported to the laboratory immediately, and stored at 4°C.

2.2. Isolation of BPA-resistant bacteria from Effluent of thermal-paper industry

The medium for LB agar plates was prepared by dissolving 1g NaCl, 1gm tryptone, and 0.5gm yeast extract in distilled water, pH was adjusted to 7 – 7.2, and made up to a final volume of 100 ml with distilled water. To this, 1.5 gm of agar was added, mixed well, and the medium autoclaved at 121°C at 15 lb/inch^2 pressure for 15 minutes. For isolation of BPA-resistant bacteria, 0.1ml of the sample from the effluent was taken. The mixing zone was spread on Luria-Bertani (LB) agar plates containing BPA at a concentration of 5mM. Growth of bacterial isolates was checked after 24 hrs of incubation at 37°C. Isolated colonies were picked and pure cultures of these prepared by repeated pour-plate technique [10], [11]. The bacterial colony showing the highest levels of degradation of BPA were designated as Strain 1. and the colony was identified and characterized by morphological, biochemical and phylogenetic studies.

2.3. Study of BPA resistance activity in nutrient agar medium

To determine BPA resistance, Strain 1 (Bacillus sp) was inoculated into nutrient agar medium containing BPA at concentrations 1mM to 30mM. Cells were cultivated in a Erlenmeyer flask (with a capacity of 500 ml) with 100 ml of medium at 35°C with agitation speed of 120 rpm in a shaker. Growth of the bacterial species was monitored by measuring optical optical density at 650 nm (OD_{650}) at specified time-intervals using a Shimadzu UV-Visible double-beam Spectrophotometer. Media were solidified if necessary by the addition of agar at a concentration of 1.5% (wt /vol). Strain - 1 was streaked on nutrient-agar plates containing BPA, and appearance of growth was taken to confirm the presence of BPA resistance activity [5], [10], [12], [21].

2.4. Study of BPA degrading activity in basal salt Medium

Strain-1 was grown in nutrient-agar medium at different concentrations of BPA and transferred to Basal Salt Medium (BSM) with following composition KH_2PO4 - 1.0gm, K_2HPO_4 - 1.0gm, $(NH_4)_2SO_4$ - 1.0gm, NaCl - 0.1gm, $MgSO_4$ - 0.2gm, $MnSO_4$ – 0.05gm, $FeCl_3.7H_2O$ –0.05gm, $CaCl_2.2H_2O$ - 0.5gm, BPA (various concentrations) and distilled water -100ml [20]. Cells were cultivated in a Erlenmeyer flask (with a capacity of 500 ml) with 100 ml of medium at 35°C and with an agitation speed of 120 rpm in a shaker. Growth was monitored at all concentrations of BPA at specified time-intervals by measuring optical density at 650 nm using UV-Visible spectrophotometer. Media were solidified if necessary by the addition of agar at a concentration of 1.5% (wt /vol) and the appearance of growth was taken to confirm the presence of BPA resistance activity [5], [20], [21].

2.5. Estimation of bacterial cell mass

The Optical Density (OD) of the culture broth at 650 nm was measured periodically using Spectrophotometer, and when this reached a value of 1, a sample of the culture was withdrawn from the flask and centrifuged at 4000 rpm for 20 minutes. The supernatant was decanted; the pellet re-suspended in de-ionized water and the cell suspension was re-centrifuged. The supernatant was again decanted, the pellet was suspended in a small volume of de-ionized water and the suspension of cells transferred onto a pre-weighed 0.45-µm filter. The filter was then dried in an oven, cooled in a desiccator at room temperature, and re-weighed till a constant weight was obtained. The difference between the final constant weight and the pre-weighed filter were used to estimate Cell Dry Weight (CDW). An optical density of one unit corresponds approximately to 1gm / liter of dry cell mass [22].

2.6. Study of growth kinetics in BPA degradation-Theoretical analysis

The study of growth kinetics is a valuable tool in biotechnological experimentation. Specific growth rate was calculated from the increase in cell mass over a period of time. The specific growth rate in the exponential phase is calculated as follows:

$$\mu max = K = \ln\left(\frac{mt2}{mt1}\right) \div t2 - t1; t2 > t1 \quad (1)$$

Where μ_{max} = Maximum specific growth rate

K = Growth-rate constant

m_t = biomass at different time-points in the exponential phase (eg. t1 and t2)

The idea of microbial growth kinetics has been dominated by an empirical model equation (2) originally proposed by Monod. The Monod model introduced the concept of a growth limiting substrate as well as the equation applied in to growth kinetics experimental data

$$\mu = \mu max.S/Ks+S \quad (2)$$

Where

μ = specific growth rate (h^{-1})

μ_{max} = maximum specific growth rate

S = residual substrate concentration

K_s = substrate saturation constant

(i.e. substrate concentration at half μ_{max}). In the Monod model, growth rate is related to the concentration of a single growth-limiting substrate through the parameters μ_{max} and K_s. All kinetic parameters were determined by non-linear regression analysis with MATLAB [19], [22].

2.7. Analysis of BPA degradation by High Performance Liquid Chromatography (HPLC).

BPA (25mM) was added to BSM as a sole carbon source. The isolate (*Bacillus sp*) was cultivated in a 500-ml flask with 100 ml of medium at 35^{o}C and with an agitation speed of 120 rpm in a shaker. Samples were withdrawn periodically (after 0, 3, 5 and 7 days of growth) and centrifuged at 9200 rpm for 10 minutes at 4^{0}C. The resulting supernatant was filtered through 0.22-µm Millex filter paper for removal of insolubles. The filtrate was used for the quantification of BPA and its degradation products by HPLC [8], [11], [20], [23].

The HPLC system used in the study has the following configuration: Waters M515 HPLC pump, gradient system, a Spherisorb column of dimensions 250mm x 4.6 mm, and a UV / Visible detector; besides, an Empower 2 Personal software was used for the HPLC data processing. The C_{18} reversed-phase column (dimensions 150mm X 4.6mm X 5µm) was equilibrated with a 30% acetonitrile solution, and an isocratic elution flow rate of 1ml /minute was applied. Samples were applied to the equilibrated column and eluted with monitoring of the absorbance at 280nm. The peak area was taken for calculation [8], [11], [15], [20], [23].

2.8. Analysis of BPA degradation activity using Gas Chromatography-Mass Spectroscopy (GC –MS)

The *Bacillus sp* was grown in 500-ml mineral medium at a concentration of 20 mM of BPA. After incubation, 50 ml of the culture broth was withdrawn and centrifuged for 15 minutes at 10,000 rpm. The supernatants were shaken with 3 x 50 ml of peroxide-free diethyl ether for 5 minutes. The pH was adjusted to 9.0 with KOH, and to 7.0 and 2.0 with HCl. The extracts were then dried with Na_2SO_4 and reduced to 0.2 ml by rotary vacuum evaporation (40^{o}C, 850 rpm).

The extracts were dissolved in acetonitrile, derivatized by addition of 50µl of N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA), and incubated for 20 minutes at 70^{o}C. After cooling down to room temperature, the samples were analyzed by GC-MS [24]. A Shimadzu QP 2010 plus series Kyoto, Japan, gas chromatograph – mass spectrometer (GC-MS) installed with an RTX-5MS column (length, 30m and inner diameter, 0.25mm) was used for structure determination. The GC conditions were as follows: helium as carrier gas at a flow rate 1.0ml /min; an injector temperature of 280^{o}C; oven temperature initially held at 60^{o}C for 5 minutes, increased to 180^{o}C for 3 minutes, further increased to 250^{o}C for 1 minute, and finally increased to 280^{o}C for 1 minute.

The GC-MS interface was maintained at 260^{o}C with 57.4kpa. of pressure. Mass spectrum analysis was performed at electron energy of 70 eV; the mass charge m/z started at 40 and ended with 400 m/z over a scanning duration of 0.50 second. The structures of the degradation products were confirmed by comparing fragmentation patterns of the mass spectra with those of authentic compounds using NIST library [24].

2.9. Factors affecting BPA Degradation

To characterize the effect of temperature (25, 30, 35, 40 and 45^{o}C) and initial pH (5,7,9,10 and 11) the autoclaved nutrient medium (100 ml) in culture flasks with a capacity of 250 ml was supplemented with BPA at a concentration of 20mM and inoculated with a log-phase bacterial culture (*Bacillus sp*). The flasks were agitated at 120 – 180 rpm, and samples were withdrawn periodically and the optical density were measured at 600 nm using UV-Visible double-beam Spectrophotometer [17], [25].

3. Results and Discussion

3.1. Identification and Characteristics of the Strain

A strain with BPA-degrading ability, isolated from the effluent of a thermal paper industry, was identified as *Bacillus subtilius* (99% Similarity with species) based on 16s rRNA, morphological, physiological and biochemical analyses. The primers which were used to amplify 16SrRNA gene were Pf:5^I–AGAGTTTGACCTGGCTCAG-3^I and Pr-5^I-ACGGCTACCTT-GTTACCGACT- 3^I.

The nucleotide sequences coding for the 16s rRNA gene of *Bacillus sp* have been submitted to Gene Bank with the Accession Number of KC197028. Figure-1 showed that comparisons of the phylogenic tree based on the 16s rRNA gene sequence over 806 bases revealed a relationship between different members of the genus *Bacillus sp.* and the bacterial isolate, BPA DEG.

Figure-1 Phylogenic tree of *Bacillus subtilius* by Neighbor-Joining method

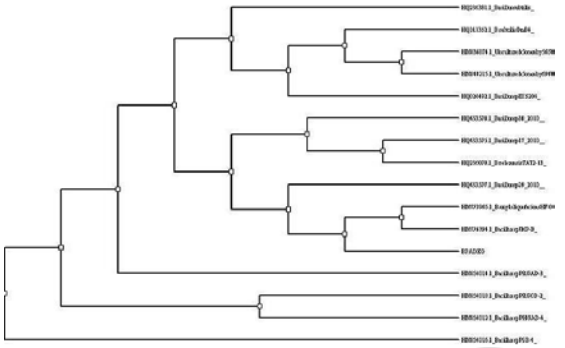


Table 1. Morphological and biochemical characteristics of Strain 1

Morphological Studies	
Shape	Rods
Gram staining	Gram-positive
Endospore	+ ve
Motility	+ve
Biochemical Studies	
Catalyse production	+ve
Oxidase production	-ve
Casein hydrolysis	-ve
Gelatin hydrolysis	-ve
Starch hydrolysis	+ve
Lipid hydrolysis	-ve
Indole production	-ve
Methyl red test	-ve
Voges-prosauker test	-ve
NO_3 test	+ve
Citrate test	-ve

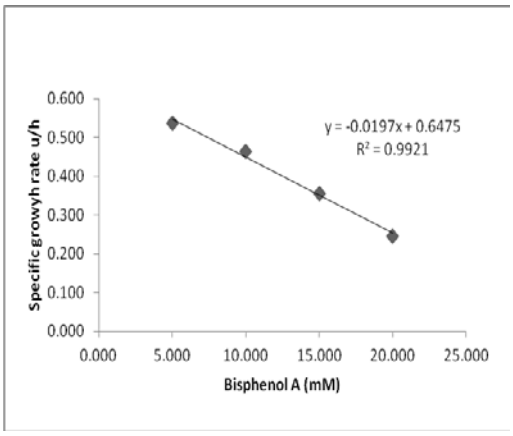
Fermentation studies	
Glucose	Acid production
Lactose	_
Sucrose	Acid production
Fructose	Acid & Gas production

The sequences were retrieved from the NCBI database and the tree drawn using the Neighbour-joining method. The cells were rod-shaped, gram-positive and motile; the isolate registered positive for starch hydrolysis and catalase production and showed negative results for oxidase activity; besides, results were also negative for hydrolysis of casein and gelatin (Table-1) [26], [27].

3.2. BPA-resistance activity in nutrient agar Medium

The *Bacillus sp* was able to resist BPA up to a concentration of 30 mM. The presence of growth confirmed the BPA resistance [5], [20]. Dry cell weight was determined at regular time intervals, and this was 0.32g/l for the *Bacillus* sp. Figure-2 showed a plot of the maximum specific growth rate (μ_{max}) on BPA concentration, indicating that increasing toxicity due to BPA decreases the microbial population, as evidenced by the linearly decreasing growth rate.

Figure-2. Specific growth rate profile of BPA degradation by *Bacillus subtilis* in nutrient agar medium



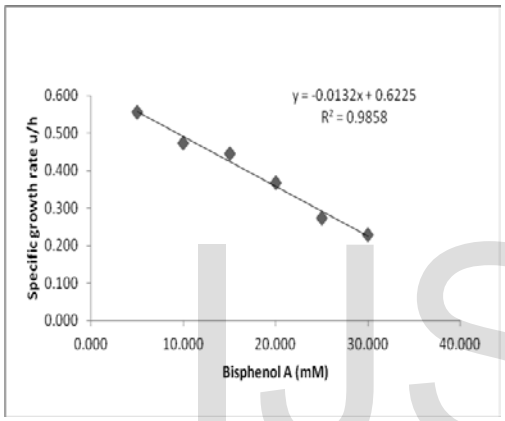
3.3. BPA-degrading activity in basal salt mineral Medium

Bacillus sp was able to degrade BPA up to 20 mM. The presence of growth confirmed the BPA resistant activity [12], [20]. Dry cell weight was determined at specified time-points. P.monteilli has

been reported to degrade 500 ppm of BPA within 10 days of incubation in BSM medium [25].

Likewise, Lee *et al.*, [20] have reported that 100 mg/ml of BPA was degraded within 6 hours in MYPG medium (Figure-3). *Streptomyces sp* degrades 1mg/ml of BPA within 3 to 4 days [8]. Therefore, in the present study, it was observed that 20mM of BPA disappears upon 9 days of incubation with *Bacillus species*, and the results of the present study corroborates with the results of previous study reports on BPA degradation.

Figure-3. Specific growth rate profile of bisphenol degradation by *Bacillus subtilius* in basal salt agar medium



3.4. Growth kinetics on BPA Modeling kinetics of growth

In order to determine the growth kinetic parameters, the specific growth rates (μ) of the culture at different BPA concentrations were calculated as per the following relationship:

$$\mu = \frac{1}{X} \times \frac{dX}{dt} \quad (3)$$

Where

X is biomass concentration at time

t and μ is the specific growth rate

(h^{-1}) calculated from the slope of the linear exponential growth curve

(N2/N1) versus time (t2/t1).

The BPA concentrations used in the BSM medium were in the range of 1 – 25 mM and 1–35mM in nutrient medium. The Monod Model Equations were solved using nonlinear regression methods by MATLAB software. The kinetics of growth obtained from these models, along with the coefficient of determination (R^2), were shown in Table - 2. From these experimental data, it was inferred that, the Monod Model fits to the BPA degradation profile.

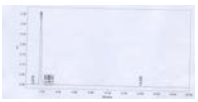
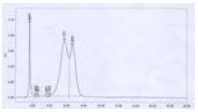
Table 2: Specific growth rate kinetic parameters obtained for BPA degradation by *Bacillus sp*.

Monad Model	Culture Medium	
	Nutrient Medium	BSM Medium
μ_{max} (h^{-1})	0.556	0.538
Ks (m*M*)	23.91	25.72
R^2	0.98	0.99

3.5. HPLC analysis of BPA and its metabolites

The culture medium was withdrawn aseptically on the initial, third, seventh and ninth day of incubation for HPLC analysis. In the control sample (Table -3), BPA, had a retention time of 1.720 minutes. and with a peak area percentage as 100.

Table – 3. HPLC analysis of bisphenol A and metabolites obtained after degradation of bisphenol A by *Bacillus subtilius.*

Time (Incubation days)	Rt (min)	Area (%)	Absorbance Spectrum (HPLC)
Initial(24)	1.725	100	
Third day(72)	1.725	93.3	
Seventh day(168)	1.684	52.84	
Ninth day(216)	1.677	11.33	

The sample analysed by HPLC after 3 days of incubation showed a major peak at 1.725 minutes. whose area percentage was 93.3; besides this, other peaks with retention times of 2.388, 2.760 and 14.026 minutes were obtained, corresponding to the products of degradation of BPA. Likewise, after 7 days of incubation, a peak with a retention time of 1.684 minutes. and an area percentage of 52.84 was

appeared. The sample withdrawn on the 9th day showed a peak at a retention time of 1.677 minutes. whose area percentage was 11.33; apart from these, other peaks, corresponding to degradation products of BPA, appeared at retention times of 2.403, 3.691, 5.737 and 6.683 minutes.

These results clearly indicated that BPA, at concentrations of up to 20mM, could be degraded to simpler compounds by *Bacillus sp.* it has been reported that a gram-negative aerobic bacterium degrades 0.5% of BPA in basal salt medium [10].

It has been also reported that BPA was degraded by a gram-negative bacterium [9]. Two fungi namely *Heterobasidium insulare* and *Stereum hirsutum*, cultivated in shallow stationary culture (SSC) in YMPG containing 200 ppm of bisphenol A, were shown to remove 68 to 77% of BPA. By HPLC analysis after the 3rd day of incubation; and on the 7th day only a trace amount of BPA was found to remain [20]. It has also been reported that degradation of 115 µg/l of BPA in LB medium was by *Sphinogomonas sp.* within six hours [11]. Further, it has been reported that the white rot basidiomycetous fungus, *Plerutous ostreatus*, degrades 0.4 mM of BPA, with the appearance of several intermediates, as evidenced by HPLC analysis [28].

Total heterotrophic bacterial populations were degrading BPA in river was ascertained by HPLC, and 0.5mM of BPA was degraded within 9 hours by *Sphinogobium yanoikuyae*, strain FM2, isolated from river water. HPLC analyses have shown that 160µm of BPA was completely degraded by *Pseudomonas sp.* within 10 days of incubation [14].

Cupriavidus basilensis JF1 degrade 0.2mM of BPA within 23 days in mineral medium upon stimulation with phenol. This organism could also utilize phenol, isopropyl phenol, ethanol, methanol, acetone and other aromatic compounds [24]. It has been concluded that, BPA was not found to be easily degraded; likewise, many authors have reported on BPA degradation but at low concentrations [12].

The results of the present study clearly demonstrated that 20 mM of BPA could be degraded more effectively by the isolated *Bacillus sp* than by the other species of bacteria reported thus far.

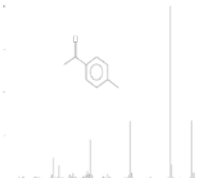
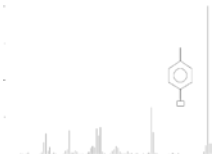
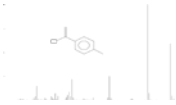
3.6. Analysis of BPA metabolites using GC –MS

To investigate the degradation pathway of BPA the organism (*Bacillus sp*) was inoculated into BSM medium. Many reports are available stating that BPA underwent a major and minor metabolic pathway of degradation pattern [9], [10].

Three different metabolites were recognized in the GC-MS spectrum which could be identified by comparisons with the known authentic compounds at corresponding retention times, namely, 4-hydroxyacetophenone (10.68 min.), hydroquinone (12.378 min), p-hydroxybenzoic acid (15.392 min) and BPA (28.378 min) (Table - 4).

The major part of BPA is cleaved in some way to yield p-hydroxyacetophenone and p-hydroxybenzoic acid which are primary sources of carbon and energy for the cell. BPA also undergoes a major metabolic pathway of degradation.

Table-4. Mass spectrum analysis of bisphenol degradation products of by *Bacillus subtilius*

Metabolites	Retention time (min)	Chemical structure /MW (m/z)	Mass spectrum
4-hydroxy-acetophenone	10.68	C_8H_8O/ 105	
Hydroquinone	12.378	$C_6H_6O_2$/ 110	
4-hydroxybenzoic Acid	15.392	$C_7H_6O_3$/ 121	
Bisphenol	28.372	$C_{15}H_{16}O_2$/ 213	

The carbon-carbon cleavage on the BPA ring leads to the removal of the phenol moiety and results in

the formation of 4,2- (propanol) phenol , the compound was not identified in the present study, but the fact of BPA undergoing this path of degradation reactions was suggested [9], [10]. Again, there was the removal of the CH_2O group from 4,2- (propanol) phenol to form 4-hydroxyacetophenone (m/z), and the same type of removal on 4-hydroxyacetophenone leads to 4-hydroxybenzoate. An oxidation step on the previous compound forms p-hydroxybenzoic acid (m/z), followed by a reduction reaction on p-hydroxybenzoic acid that leads to hydroquinone(m/z). Finally, all metabolites undergo a mineralization process and are converted to simpler compounds by the activity of microflora (*Bacillus subtilis*) [11], [17], [24], [29].

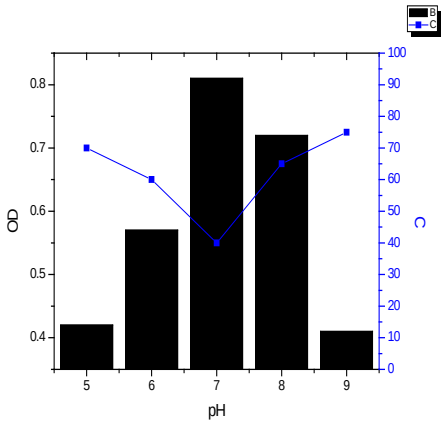
It has been detected that BPA undergoes both the major and minor routes of metabolite formation, the the major route being 1,2 – bis (4-hydroxyphenyl)2-propanol, 4-hydroxybenzoate and 4-hydroxyacetophenone; and the minor route 2,2 bis (4-hydroxyphenyl) 2-propanol and 4-hydroxyphencyl alcohol[9], [10]. Further it has also been reported that BPA is cleaved to 4,2-propanol phenol and p-hydroquinone. The splitting of 4-(2-proapanol) -phenol leads to 4-isopropenylphenol and 4 – hydroxyacetophenone. This has been converted to p-hydroxybenzoic acid which was then mineralized. The major intermediates were 4 –hydroxyacetophenone and p-hydroxybenzoic acid. BPA was metabolized to form three major intermediates which were p-hydroxyacetophenone, p-hydroxybenzaldehyde and p-isopropenylphenol [24].

Pathway I involves the oxidation of p-hydroxyacetophenone to form p-hydroxybenzoic acid. Pathway-II involves the mineralization of p-hydroxybenzaldehyde in to CO_2 with the cell mass. In pathway III, p-isopropenylphenol is oxidized to from p-hydroxybenzoic acid, and finally, all the intermediates are mineralized. It has also been reported that BPA was degraded by *Sphinogomonas sp*., strain TTNP3, and that, the major intermediates were hydroquinone, acetophenone, quinol, alkyloxyphenol and nonanol [17], [29].

3.7. Factors affecting BPA Degradation

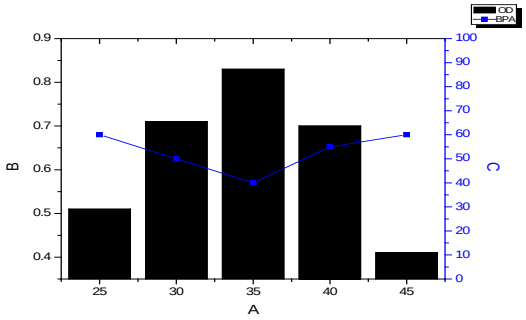
Temperature and pH are important factors that have an effect on microbial degradation. In the present study, initial pH was adjusted to 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. As shown in **Figure- 4,** BPA degradation as well as cell growth increased with increase in pH, reaching a maximum at pH 7.0, and decreased thereafter.

Figure – 4. Effects of pH on Bisphenol A degradation



BPA was effectively removed from wastewater using electrochemical oxidation at high sodium chloride and pH [30]. Generally, bacteria need a physiological pH inside their cells like all other living organisms [17]. In the present study, the *Bacillus sp.* could degrade BPA over a wide pH range (from 5.0 to 9.0). The maximum BPA degradation and cell growth were observed at pH 7.0 (Figure-4). The percentage of removal of BPA was calculated by HPLC methods described by Erica danzyl [15]. The fact that, BPA degradation and cell growth were found to be slow in acidic and alkaline conditions suggested that, the BPA-degrading enzymes have their optimal activity at neutral pH. **Figure-5** showed the effect of temperature on BPA degradation.

Figure-5. Effects of temperature on bis Bisphenol A degradation



(NB. X-Axix - % of BPA Residues)

BPA degradation as well as cell growth increased with increase in temperature and reached the maximum at 35°C. The fact that effects of temperature on BPA degradation and cell growth were more acute at lower than at higher temperatures suggested that, the growth and BPA-degrading activity of cells may be inhibited at lower temperatures [17]. BPA degradation by *Bacillus sp.* was evaluated (about 60% of BPA was removed) at different temperatures and pH. The approximate

temperature and pH at which the efficient removal of BPA were 35°C and 7.0, respectively. Zhan *et al.*,[17] have reported that, temperature and pH are also important factors for BPA degradation of *Chromobacter xylosoxidans*, strain B-16, whose temperature and pH optima for BPA degradation were 35 °C and 7.0, respectively [31], [32], [33].

4 .Conclusions

Bacillus subtilis is able to resist BPA in nutrient agar medium, thereby utilizing it as a sole carbon source in BSM medium. Furthermore, it as shown to have excellent potential for degrading BPA under in- vitro conditions (laboratory conditions). These results suggested that, environmental pollution and toxicity by BPA could be eliminated by biological treatment and this has been ascertained by the HPLC data and kinetics models.

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