

Dynamic of Bisphenol A biodegradation in laboratory conditions

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ABSTRACT

Plenty of synthetic substances including multi-component petroleum products, persistent organic pollutants (POP), pharmaceutical metabolites, must be removed both from the soil, fresh and ground-water. Presence of POPs lead to degradation of multi-species water or soil ecosystems. Many toxic substances are spread in environment by daily activity of common people. Simple shopping generates only some shop receipts for each family or simple man. Due to common use of thermal paper for electronic cash confirmations or shop recipe printouts, some toxic compounds including Bisphenol A is transferred to environment and human bodies. The aim of this study was to compare the possibilities of microbiological degradation of Bisphenol A by *Bacillus thuringiensis* and *Aspergillus niger* in laboratory test. The degradation tests have been carried for 10 d in the dark and temperature of 20°C. Two growth mediums were tested. Shredded shop recipes (1:50 m/m) was enriched by 0.2 g of bacteria *B. thuringiensis* powder or 0.2 g of fungi *A. niger* powder and incubated with rotation. Content of Bisphenol A (BPA) was determined with gas chromatography with mass spectrometer (GC-MS/MS) method. Low amounts of BPA were decomposed in both mediums. Degradation of BPA was weak in case of both tested organisms and not exceed 67% after 10 d.

Keywords: Bisphenol A; Biodegradation; Thermal paper; Shop receipts; *Bacillus thuringiensis*; *Aspergillus niger*

1. Introduction

Bisphenol A (BPA) is an organic synthetic compound belonging to the group of diphenylmethane derivatives and bisphenols [1]. Production of BPA containing goods is very high. Especially packaging materials, toys, dental materials, healthcare equipment and thermal paper which is the second (after water and food), most common source of BPA [2]. Due to its phenolic rings BPA has a possibility to interact with estrogen receptors (due to its structural similarity to 17 β -estradiol) and to act as agonist or antagonist *via* estrogen receptor (ER) dependent signaling pathways [3]. Therefore, BPA has been shown to play a role in

the pathogenesis of several endocrine disorders including female and male infertility, precocious puberty, hormone dependent tumors such as breast and prostate cancer and several metabolic disorders including polycystic ovary syndrome (PCOS) [2,4]. Also, wildlife is changing under BPA pressing. There are presence of development inhibition, malformations, and changes in the reproductive system [5]. Due to its toxic character, this compound should be fast removed from waste stream to avoid environment pollution possibility. Especially high content of BPA was shown in landfill effluents (17.2 mg/dm³) and shop recipes, money transfer confirmations with use of thermal paper which contains 6–14 g·BPA/kg [3,6]. Presence of BPA was confirmed in many

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samples of water, soil, plants, animal and human tissues or human urine and landfill leachates [7–9]. Due to permanent growth of BPA production and growing concentrations in environment, a separate collection of BPA-rich waste type is a way for minimization of BPA elution [4]. Several data show persistent nature of bisphenols, what generate a risk of long-live of it in environment [10]. Degradation procedures with using ozone, UV radiation was no effective and this type of processes can't be introduced in practice. On the other hand, advanced oxidation processes (AOP) processes have a potential to quick degradation of BPA. The AOP procedures with UVC/HOCl, UVC/S₂O₈²⁻, and UVC/H₂O₂ gives a positive results, with the most effective of UVC/HOCl and low amount of toxic by-products, also good from economical point of view [11]. Also, ultrasounds and Fenton's reagent were compared in BPA degradation. Oxidation with Fenton's processes were slightly more effective than ultrasonication in case of total organic carbon (TOC) and chemical oxygen deman analysis as a base of degradation stage [12]. One of more effective biodegradation processes is use of different fungi or bacteria species. Especially effective in BPA decomposition was fungi species *Phanerochaete sordida* YK-624, or *Sphingomonas* sp. YK5 [13,14]. Also various types of bacteria are able to quick BPA degradation. Good results were obtained with *Ralstonia eutropha* [15], *Pseudomonas putida* [16] and *Bacillus thuringiensis* GIMCC1.817 [1].

B. thuringiensis is a gram-positive bacteria which produces a toxic cry proteins very effective for some species of insects control in plant production [1]. *B. thuringiensis* cells are mobile, fully ciliated, spore rods 2–5 µm long and approximately 1 µm wide, usually arranged in pairs or short chains. Cry toxins are usually defined as parasporal protein crystals with insecticidal activity. On the other hand, the Cyt toxins, include para spora IBt proteins that exhibit hemolytic activity. It was found that the present toxins are highly specific in action in relation to the target insects, harmless to humans, vertebrates and plants, and completely biodegradable [17,18]. Moreover, this species is useful for some valuable compounds production, for example, L-methionine useful on animal nutrition [19]. In experiments with *Rhodococcus equi*, degradation exceed 60% but only in case of lower concentration (5 mg/dm³) of BPA in solution. Strong correlation was observed in degradation speed and BPA concentration, but even in highly concentrated solutions (50 mg/dm³) after 2 h reached 20% [20]. Excellent results of BPA biodegradation speed was achieved in case of use an engineered *Shewanella oneidensis* which produce effective Cytochrome P450 and ferredoxin. At low concentration (10 µg/mL) biofilm of this species can degrade BPA in 10 min [21].

As an important model microbe, *B. thuringiensis* contains enzymes that cleave benzene ring-containing pollutants, including high concentrations of dimethyl phthalate [22], fipronil [23] and triphenyltin [24]. Therefore, the aim of this study was to compare the possibilities of microbiological degradation of Bisphenol A by *B. thuringiensis* and *Aspergillus niger* in laboratory test.

1.1. BPA environmental and human risk

BPA has been recognized as a compound playing a role in the development of various diseases dependent on

endocrine hormones. Among other things, it negatively affects the fertility of women and men, causes diabetes, obesity, premature puberty and the development of hormone-dependent cancers, including breast, prostate and colon [26,27]. In addition, it causes diseases such as PCOS [2]. Presence of BPA significantly change the bacteria cell membrane permeability. In final effect it change metabolism of amino acids and proteins and also carbon, purine, pyrimidine and fatty acids [1]. The main sources of BPA for humans are: drinking water, food (dietary sources) and thermal paper used for shop receipts and debit card confirmation (non-dietary source). Due to the data mentioned above, the maximum acceptable daily intake of BPA was established to 4 µg/kg including all possible sources [5].

1.2. Bisphenol A characteristics

Bisphenol A (BPA or diphenylolpropane) (Fig. 1) is a popular compound in polymer industry. Mostly it is use in plastics production as plasticizer. A lot of BPA is used for PVC, PC and epoxy resins and vinyl ester resins manufacturing. A lot of BPA is also used as a developing agent in thermal paper (TP) due to thermal printing popular technology of a lot of printouts in public sector: shop receipts, museum or parking tickets and debit/credit card payment confirmation [25].

2. Materials and methods

Shop receipts (SR) collected from different local shops were shredded in small paper shredder. Obtained sample (about 3 kg) was mixed manually and stored in the dark and room temperature prior to use. For Bisphenol A analysis in SR material, a solid-liquid extraction with acetone/hexane (1:1 v/v) and acetonitrile with an automatic extractor feXKA® was made. Extracts was evaporated to 1 mL, dried with anhydrous Na₂SO₄ and analyzed by gas chromatography (GC-MS/MS). Water solubility of BPA was measured in leaching procedure. Deionized water and SR was used in 1:50 (m/v) ratio in dark for 24 h at 20°C in three independent replicates.

Biodegradation test was carried out in glass Erlenmeyer flasks in the dark at 20°C with NP and LC mediums. Bacteria powder with *B. thuringiensis* and *A. niger* were used as a core degradation organism. The 2.0 g of receipt samples was incubated in 200 cm³ of two different growth media: NP (NH₄NO₃ 2.6 g; 0.52 g K₂HPO₄/dm³) and LB (peptone, 10.0 g; yeast extract, 10.0 g; NaCl, 5.0 g; H₂O/dm³) [1]. Both media were pasteurized prior to use. Both media were enriched with 0.2 g of *B. thuringiensis* var. *Kurstaki* ABTS 351 bacteria powder (insecticide commercial product). For comparison in NP.medium (with addition of sacharose 1 g/dm³) was enriched with 0.2 g mycelial hyphae of dry *A. niger* samples (taken from natural environment). Three types of samples were obtained: NP_BT, LB_BT – with *B. thuringiensis* and NP_AN – with *A. niger*. Experiment was conducted in the dark with stirring (80 rpm). Samples were additionally aerated twice a day for 1 min. Samples for BPA analysis were taken after 1, 3, 5 and 10 d. The total content of nitrogen and total content of organic carbon was determined with automatic Jena C/N analyzer. The pH value was determined in

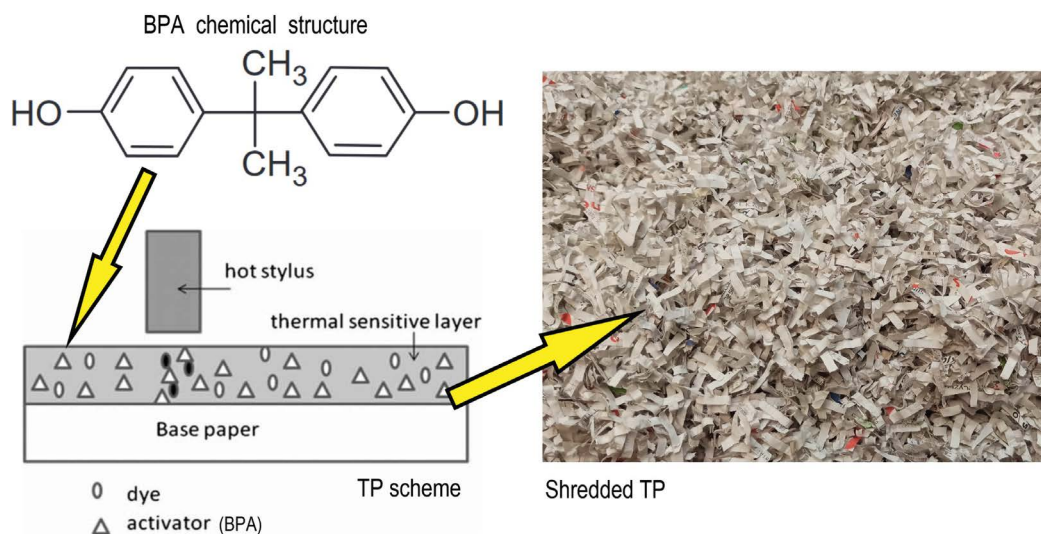


Fig. 1. Bisphenol A structure and role as dye activator in thermal paper [25] changed.

H₂O using 1:10 solution. The treatment and control samples were divided to liquid (supernatant) and solid (SR) part. Liquid part was extracted by ultrasound assisted liquid–liquid extraction with 15 mL hexane. Solid part (SR) was dried and extracted with acetone/hexane (1:1 v/v) and acetonitrile with an automatic extractor. Obtained samples were concentrated to 0.5 cm³, and analyzed by gas chromatography with mass spectrometer (Varian 3900 GC-MS/MS). The GC-MS analyses were operated in EI mode at 70 eV. The source temperature was 250°C and emission current 300 μA. The gas chromatograph was equipped with a capillary column VF-35MS (length of 30 m and internal diameter of 0.25 mm) with 0.25 μm film thickness. The temperature was programmed from 105°C (3 min) to 120°C at 12°C/min and then to 300°C at 15°C/min, finally holding at this temperature for 2 min. For quantification a BPA standard DRE-C10655500 was used. Quality control was performed with using a laboratory solvent blanks, a matrix blank. All used chemicals were chromatography-grade.

3. Results and discussion

Characteristic of raw growth media ad enriched with bacteria or fungi powder are shown in Table 1. Water extract with a lot of TOC and low total nitrogen is not a good medium for bacteria, but was only a control sample for check of nitrogen and carbon migration from TP to solution.

3.1. Characteristics of thermal paper BPA content

Thermal paper (SR) extract (Fig. 2) used in experiments contain high amounts of different organic compounds (red arrows peaks) which appears on chromatogram before Bisphenol A retention time (RT 15.07). BPA is a thin peak marked with yellow arrow peak and “1A” flag. The highest peaks was observed in case of 4-(2-naphtyloamino) phenol (CAS: 93-45-8, RT 14.8 min). Concentrations of this compound was very high (due to its compatibility even

Table 1
Basic characteristics of investigated medium samples (*n* = 3)

	Total nitrogen (mg/dm ³)	Total organic carbon (mg/dm ³)	pH	C:N
Shop receipt water extract	4.33	275.8	7.18	63.7
NP	942.4	9.95	5.13	0.011
NP.BT	974.6	355.5	5.16	0.365
NP.AN	3,032	740.4	5.03	0.244
LB	1,713	4,281	6.91	2.499
LB_BT	1,759	4,620	6.88	2.627

with strong oxidizing agent) also in LB and NP medium extracts obtained during experiment. Mean peak area of this compound was 5.84 and 3.99 times higher than BPA peak in NP and LB, respectively. Exact concentration of RT 14.8 min peak compound is unknown, but observed very high concentration can retard BPA biodegradation speed, if this compound be used as carbon source for microbial populations or be a factor which inhibit enzymes activity. True environmental impact of it was not investigated (no literature data). In high concentrated extracts, an interesting compound was found (RT 14.12 peak 3 (Fig. 3). It was identified as 6,6'-biquinoline (CAS: 612-79-3) and reports suggest antimicrobial activity of its derivatives [28]. Peak 2 compound is diphenylsulfone (CAS: 127-63-9), which toxicity is not high [29]. Peak 1 is an Ursol Blue Grey OM (CAS: 6219-89-2) with low water solubility and unknown water organisms toxicity. However, water solubility of this compound is low (less than 1 mg/cm³), it is very dangerous in natural environment which may cause long lasting harmful effects to aquatic life. Content of BPA in investigated SR samples was medium (5.65 ± 0.59 g/kg). Due to low solubility, obtained water extract contain only 1.9 μg-BPA/dm³ and that was found as a factor of low degradation stage.

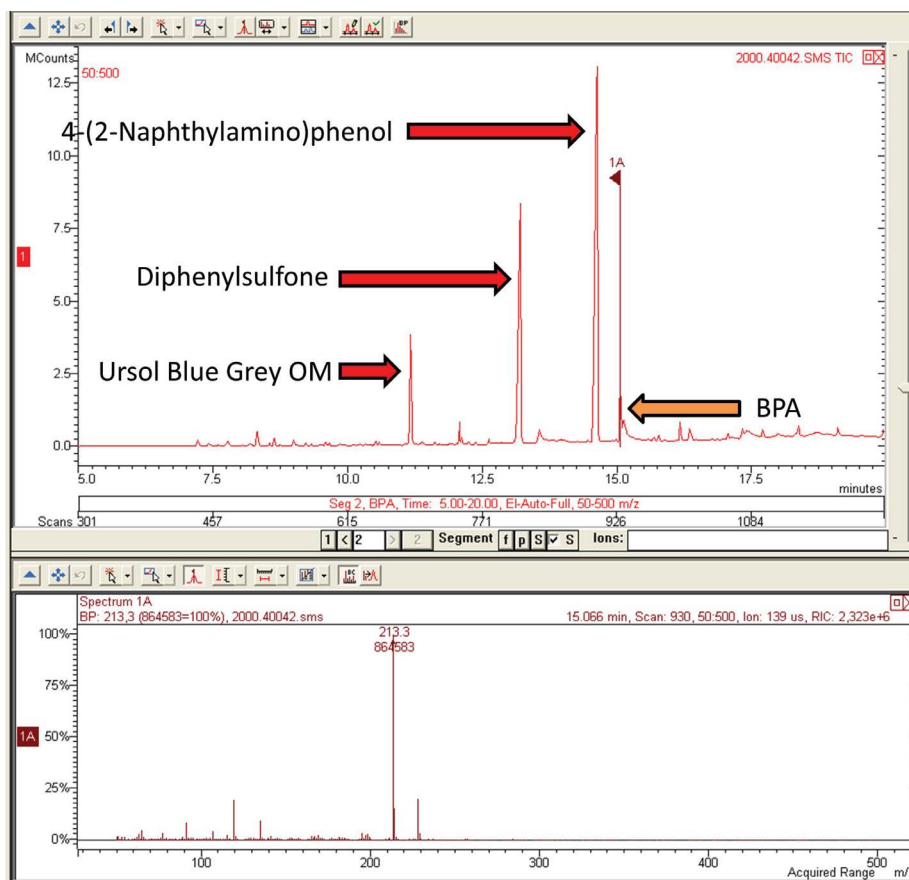


Fig. 2. Shop receipt extract gas chromatography with mass spectrometer plot with Bisphenol A compound mass bar (213.3).

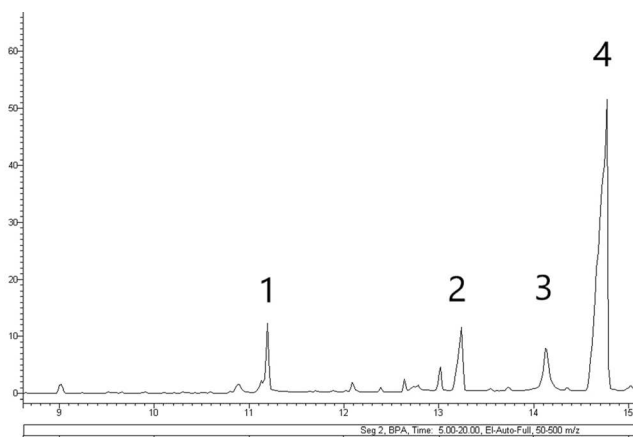


Fig. 3. Bacteriostatic compound 6,6'-biquinoline (RT 14.12) in shop receipt extract.

Degradation of BPA can be observed in presence of chemical factors like soluble Mn(II) also with H_2O_2 AOP processes or presence of microorganisms especially with external enzymes production [3,30].

Literature data indicate a significant efficiency of the BPA microbial degradation process of 85% in 24 h using a 1 μ M solution, but on the other hand only co-metabolism can be done because the bacteria consortia could not use BPA as

carbon source solely. The base of further experiments was a data about possibility of use benzene ring compounds as sole carbon nutrients, a good results [1,15]. A lot of microbial genera is ready to BPA degradation but in case of ensure specific conditions enzymatic activities can be really high. Isolated strains of *Bacillus* was able to complete BPA degradation after 60 h [5]. Processes of micropollutants degradation by *B. thuringiensis* use with light presence were not effective due to light inhibition role [31]. The degradation of BPA in the conditions of the experiment carried out here (without pre-growth and cell isolation) was weak, which indicates low microbial activity. Obtained data (Figs. 4 and 5) show concentrations of BPA in SR sample and eluate samples. As a base of degradation effectivity was lost of BPA determined in SR samples. After 24 h the highest effectivity was observed in case of LB_BT samples where almost 50% of BPA was lost what was observed also with higher intensity, in experiments with 1 μ M BPA concentration [1]. After 10 d of incubation the best results were observed in case of LB_BT (over 66% degradation) and the worst in case of NP_AN (only 38.9%). Usually about 50% of BPA degradation takes 2–3 d in aerobic conditions, what show ability of tested organism to this xenobiotic consumption [3]. On the one hand LB_BT combination can be useful for BPA pollutant removal, on the other hand low degradation stage can be a result of low pH value and to low C:N ratio. It was expected that low carbon content will increase a level

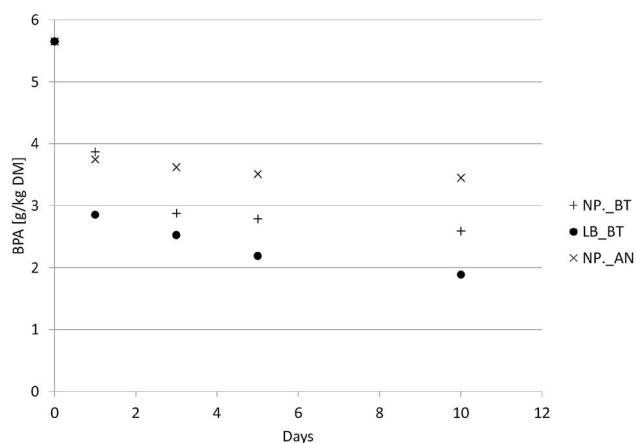


Fig. 4. Bisphenol A content in shop receipt during experiment.

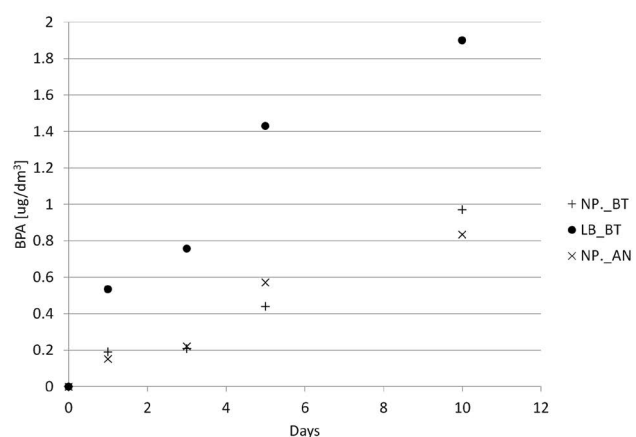


Fig. 5. Bisphenol A content in growth mediums during experiment.

of BPA consumption as a C source. Experimental data with river water spiked with BPA show high potential of degradation even in 1–3 d of aerobic incubation [16]. Experiment show that not pretreated microbial communities are not able to degrade a lot of BPA connected with SR in short time, but degradation process still exist. Much more effective is use of isolated from soil or water strains and use it only for one spiked compound [5]. It is also a result of high hydrophobicity of analyzed compound, when degradation can be mostly found on the SR surface and co-presence of other, sometimes toxic compounds in SR thermal emulsion.

On the other hand, BPA biodegradation tests are usually conducted with pre-growth bacteria population and spiked BPA as only one xenobiotic carbon source. During this experiment a special type of wastewater was made, rich in other various organic compounds. They could be a good carbon source for *B. thuringiensis* or *A. niger*, but on the one hand, some of them might blocking the active sites of degradation enzymes thus inhibiting degradation processes [4]. As an inhibition factor during BPA biodegradation process could be also high concentration of this compound on SR surface. However, an initial content was slightly lower than usually

reported in literature, but finally much more higher than usually tested in experimental conditions. In total there was 11.2 mg BPA on surface of 2 g of SR sample [3,6].

Significant concentrations of accompanying compounds like 4-(2-naphthylamino)phenol, could be the reason not only for the retardation of the BPA biodegradation process, but can be an important factor for finding new effective wastewater treatment methods. However currently (from January 1, 2020) BPA in thermal paper has been replaced with a similar compound – bisphenol S (BPS) a negative consequences of the new compound were also confirmed [32,33].

4. Conclusions

The recorded concentration of BPA in the tested TP material was lower than expected, probably as change of production processes. The obtained results indicate the degradation of BPA in the case of the LB medium used, but after 10 d of incubation it did not exceed 66% of the total content of this compound in the tested shredded SR. As a retardation factor a coexistence of other compounds (also with bacteriostatic properties) in high concentrations can be shown. The process of decomposition of Bisphenol A with the use of NP medium was not effective, probably as a result of inappropriate conditions (pH and C:N ratio) which can be a limiting factor the growth of bacteria and fungi.

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