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The effects of US/H_2O_2 processes on bisphenol-A toxicity in aqueous solutions using *Daphnia magna*

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ABSTRACT

Bisphenol-A (BPA) is one of the derivatives of phenol. Wastewater containing BPA can be a source of contamination in aquatic environments and as an endocrine disrupting chemical can cause serious hazard for human and animals. The aim of this study is to examine and compare the efficiency of ultrasonic waves (US) and hydrogen peroxide (H_2O_2) processes on BPA toxicity before and after the single (US) and combined processes (US/H_2O_2) using bioassay of *Daphnia magna* (*D. magna*). Experiments were performed at initial concentrations of 2, 5, 20 and 50 mg/L of BPA, frequency of 35 and 130 kHz, acoustic powers of 300 W and 500 W and times of 15, 30, 45, 60, 90 and 120 min. According to the probit method, LC_{50} of BPA was determined in 24, 48, 72 and 96 h. Comparison of bioassay results showed that the toxicity of the initial solution was reduced after processed with US and US/H_2O_2.

Keywords: Biphenol-A; Toxicity; Ultrasonic waves; Hydrogen peroxide; Daphnia magna; Bioassay

1. Introduction

1.1. Health importance of Bisphenol-A

BPA ($C_{15}H_{16}O_2$) (Fig. 1) is a solid with low volatility properties at normal environmental temperatures. Its solubility in water is greater in an alkaline pH (pKa 9.9–11.3) and ranges from 120 to 300 mg/L. BPA is very dangerous in aquatic ecosystems because of its endocrine disrupting effects. Even the presence of low concentration of BPA in water will impair the aquatic life by its endocrine disrupting effects. Very low doses of BPA cause cell proliferation in human prostate cancer, heart disease, diabetes (type 2) and abnormality of liver enzymes [1–5].

BPA is classified as a disrupting in hormone balance of human and animals. The estrogenic activity of this material has been demonstrated even in concentrations less than 1ng/l. Besides BPA will raise estrogenic effects in human and hormone that causes breast cancer and also is reported as anti-androgen activator which causes feminity side effects. Its amount in male and female blood is followed by reproductive disorders, endometrial hyperplasia, recurrent miscarriages, fetal genetic abnormalities and polycystic ovary syndrome [5–8].

Recently it was discovered that health hazards can occur as the result of contact with very lower dose than 0.05 mg/kg of body weight. Although regarding US EPA reports the permitted daily dosage for human is determined 50 µg/kg of body weight, but it is discovered that the estrogen receptors of the cell membrane can be irritated as a physiological response to BPA in pg/mg concentrations [8–10].

Its acute toxicity is about 1–10 µg/mL in fresh and sea water. Some effects have been reported in fish at concentration of 1.82 µg/mL, but at concentration of 0.355 µg/mL the chronic effects may occur in 60 d. The chronic toxicity for *D. magna* will be also 3146 µg/l [10–13].

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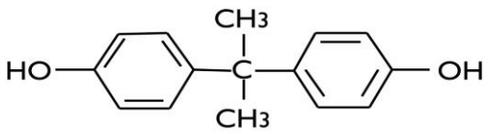

Fig. 1. Chemical structure of BPA.

1.2. The importance of ultrasonic waves

Ultrasonic waves are the simple form of the sound that human cannot understand. Oscillation with a frequency of more than 20 Hz is called ultrasonic waves. Each wave (ultra sound or hearing) is a mechanical turbulence in gas, liquid or solid that moves with a steady pace. These waves efficiently meet in our daily life such as industry, medicine and the environment [14–16].

Ultrasound waves will lead to the destruction of organic matter based on the cavitations small bubbles. High temperature and pressure during the collapse of the bubbles can cause sonolysis of organic materials and production of highly reactive radicals. The approximate temperature will be about 4000–10000°k and 300–975 bar pressure. This situation will be suitable for more sonolysis processes. The concentration of hydroxyl radicals can be more than 4×10^{-3} mol in a bubble which is 10^8–10^9 times more than other advanced oxidation processes. Sonolysis of pollutants also can cause formation of new radicals and starting other chain reactions [16–22].

Generally, generation of free radicals during sonolysis is described by the equations in the presence of dissolved oxygen in aqueous solution [22–24]:

$$H_2O \rightarrow H\cdot + \cdot OH \quad (1)$$

$$O_2 \rightarrow 2O\cdot \quad (2)$$

$$\cdot OH + \cdot OH \rightarrow H_2O + O\cdot \quad (3)$$

$$\cdot OH + \cdot OH \rightarrow H_2 + O_2 \quad (4)$$

$$\cdot OH(aq) + \cdot OH\ (aq) \rightarrow H_2O_2 \quad (5)$$

$$\cdot OH + H_2O \rightarrow H_2O_2 + H\cdot \quad (6)$$

$$H\cdot + \cdot OH \rightarrow H_2O \quad (7)$$

$$H\cdot + H\cdot \rightarrow H_2 \quad (8)$$

$$O\cdot + O\cdot \rightarrow O_2 \quad (9)$$

$$O\cdot + H_2O \rightarrow \cdot OH \quad (10)$$

$$O_2 \rightarrow O\cdot + O\cdot \quad (11)$$

$$O_2 + O\cdot \rightarrow O_3 \quad (12)$$

$$H\cdot + O_2 \rightarrow HO_2\cdot \quad (13)$$

$$HO_2\cdot + H\cdot \rightarrow H_2O \quad (14)$$

$$HO_2\cdot + HO_2\cdot \rightarrow H_2O + H_2 \quad (15)$$

$$HO_2\cdot \rightarrow \cdot OH + 1/2\ H_2 \quad (16)$$

1.3. The importance of bioassay

Bioassay is necessary for the assessment of water pollution because chemical and physical tests are not sufficient to assess potential impacts on aquatic life. For example the interaction between the chemical elements and toxicants cannot be set. Even with the extensive physiochemical characteristics of water, it is not possible to relieve its adverse effect on human. Thus, the biological effects of toxicants can be followed by bioassay. Bioassay is very valuable in impact assessment processes in water treatment to reduce the toxicity. Varieties of aquatic organisms are used for this purpose [25–27]. These organisms are susceptible to the toxicants with different extents; organisms with the similar sensitivity even are not the same in the different stages of their lives. Even organisms of the same species will act differently in contact with the same quantities of toxicants while other parameters are constant. In addition to the scientific value of the bioassay it is easy to accomplish. It also requires relatively little time and, in some cases, tests are done in less than an hour [25–29].

2. Materials and Methods

2.1. D. magna culture

D. magna (Fig. 2) is the greatest type of *D. magna* with 5 mm length. A lot of this organism can be developed in a relatively small space. *D. magna* infants have 0.8–1 mm length and can be seen with naked eye. *D. magna* are important at this stage of life for bioassay. The female *D. magna* gives birth to her first infants during 10 d in 20°C and during 7 d in 25°C. The next infants will be born 3–4 d later in 20°C and 2–3 d later in 25°C. If the medium and other conditions are favorable each time 12 infants will be born. When *D. magna* is growing in 20°C, its infants will moult after 30 h for the first time. If the temperature changes to 25°C, the time will reduce to 20 h. Infants can be born in a period of 24 h in 20°C and in 12 h in 25°C. They have low resistance to most of the matters in compare with older animals. *D. magna* is more sensitive while moulting, than the distance between two moults. One of the most simple culture for *D. magna* is plant-soil medium which should be strengthens with yeast periodically. This medium is provided from mixing 5 g of dried sheep manure, 25 g garden soil or compost mixed with sand and one liter of pond, fountain or decholorized valve water. After 2 d remaining in room temperature, it should be smoothed by filtering with a cloth which has 0.15 mm holes in it. Some small parts of the soil will pass the cloth while filtering. The smoothed liquid should stay for one week or more in a place and then the sediment parts will be discarded. To make the final culture media is a mixture of one part of filtered liquid to 6–8 parts of pond,

fountain or dechlorinated valve water. The primary filtered liquid may be kept a long time before the final media is made. Final culture can be used for either individual or for herd cultivation. For individual cultivation 100 mL of media is poured into a glass bottle, and then one *D. magna* will be released into it. One day later, 1 mL of a suspension made from 1 mg dry active yeast will be added to each bottle each 2 d. For the mass cultivation a 3.8 L glass bottle is used which should contains 3 L media and 30 mg dry active yeasts should be added to that each two days [30–35].

2.2. Growth and production of D. magna

Primary *D. magna* were caught for cultivation from its normal environment. In the first stage one *D. magna* was cultivated alone in the media when infants were born they were fed until they reached maturity and reproduction age. After that, infants of the same mother were cultivated in a mass form. Culture mediums should be exposed in the sunlight during the day, and at night the lab should be dark. It should be noted that the temperature control should be done permanently with a thermometer and it should be kept constantly 20 ± 1°C during the cultivation. pH and dissolved oxygen (DO) should be controlled during that time also. According to the tests, it was found out that pH remained almost viable, but DO level reduced which was operated with aeration. Aeration is not necessary since critical DO for *D. magna* is less than 15% of saturation oxygen in 20°C, however to ensure of proper situation an aeration pump is used weekly. Using 100 female *D. magna* cultivated individually, 300 infants will be generated daily. When females start to reproduce, young will be isolated periodically. This should preferably be at temperature of 20°C each 24 h and at 25°C each 12 h. When the primary animals grew old and the reproduction rate failed, young female will be used in fresh culture media. It should be noted that the culture should be preferably used in glass ware [30–35].

2.3. Death in witness samples

In this study, *D. magna* infants (4–5 d old) were gathered and washed three times for 5 min in diluting water. Then 9 containers were chosen with one witness sample. Ten *D. magna* were added to each container. In the next step, 5, 10, 20, 30, 40, 50, 75 and 100 mL of the sample was added to them. They were observed after 24, 48, 72 and 96 h. After recording the observations in the mentioned periods, dead *D. magna* were counted. The *D. magna* was considered as dead if it did not move after the rotation of the bottle.

Fig. 2. *Daphnia magna*.

2.4. Using of single and combined processes

2.4.1. First step: Using US

To determine the BPA removal by sonolysis process experiments were carried out at different conditions. For this purpose, the samples of 100 mL were made from stock solution and placed in an ultrasonic reactor (Fig. 3). Ultrasonic reactor characteristics are presented in Table 1. All of the experimental processes in ultrasonic stage were done in four concentration levels (2, 5, 20 and 50 mg/L of BPA),

Fig. 3. Typical reactor used for determination of toxicity in this study.

Table 1
Characteristics of US reactor in this study

Characteristics	
Rector type	TI-H-5
Country	Germany
Company	Elma
Maximum capacity	4.7 L
Dimensions of the external container	370 × 300 × 340 mm
Dimensions of the internal container	150 × 130 × 240 mm
Input power	500 W
Output power	100 W
Output intensity	2.5 w/cm^2
Frequency	130 kHz and 35 kHz
Type	piezoelectric
Diameter	5 cm
Material	steel
Weight	10.5 kg

two levels of powers (300 W and 500 W), three levels of pHs (3, 7 and 11), two levels of frequency (35 and 130 kHz) and six levels of contact times (15, 30, 45, 60, 90 and 120 min). To maintain the ultrasonic bath in the range of ambient air, water inside the bath was circulated.

2.4.2. Second step: Using H_2O_2

In this stage, samples of BPA were made in desired concentrations and pHs of the solution were modified. Then 5, 15 and 30 mg/L of 30% H_2O_2 stock were added to BPA samples and were mixed with GFL 3018 shaker in 5, 10, 20, 30, 45, 60 and 90 min to determine the toxicity of BPA by H_2O_2.

2.4.3. Third step: Using US/H_2O_2

In this stage, composing both methods was used in removal of BPA. For this purpose, first samples of 100 mL were made and placed in ultrasonic reactor. At the same time H_2O_2 were added to them in 5, 15 and 30 mg/L concentrations. The whole experimental processes in US/H_2O_2 method were done according to conditions as mentioned above.

All the experiments were performed according to the Standard Methods for the Examination of Water and Wastewater (2012) and USEPA-method 821-R-02-012. Data analysis and computation of LC_{50} were performed using pro-bit analysis. Toxicity unit was calculated by dividing 100 by LC_{50} [30–37].

3. Results and discussion

3.1. BPA toxicity test with different concentrations

Samples of BPA were made in different concentrations. *D. magna* infants were considered 3–4 d old. After preparing the solutions, infants were gathered in a container and each container contains 10 infants. The separated infants should be washed in diluting water three times; each washing time should take 5 min. After entering infants to the test solution, they were observed after 24, 48, 72 and 96 h regularly carefully. The number of non-moving animal was recorded in each observation. The non-moving animal is an animal which does not move even after rotating the container. It should be noted that non-moving animals are not specifically dead and there should be enough care in counting dead ones. *D. magna* should not be fed during the test and they will stay alive for a week without food in solutions with controlled salt.

3.2. Determination of toxicity during US and H_2O_2 processes

To determine the toxicity of remained BPA in solution after ultrasonic, H_2O_2 and the combined processes, initial samples were made from the outlet of each reactor. The concentration of BPA was 5 mg/L in the initial samples. Due to the unknown concentration of various products in the samples, eight samples were made with 5, 10, 20, 30, 40, 50, 75 and 100 volume of the initial sample for the bioassay test. The experiments were performed at neutral pH. In this step which is determining the efficiency of ultrasonic reactor in reducing the toxicity of BPA after making the samples they were put in the ultrasonic reactor and were tested in different times (optimum conditions; concentration of BPA 2 mg/L, concentration of H_2O_2 30 mg/L and pH 11). Same as the last method, *D. magna* infants which exit each 24 h in 20°C and each 12 h in 25°C, were gathered in a container. Washed in diluting water three times, each washing time should take 5 min and each container contains 10 infants. The experiment had 5–7 containers with one witness. After entering infants to the solution, observation was done periodically and carefully after 24–96 h. The number of non-moving animal was recorded in each observation. The non-moving animal is an animal which does not move even after rotating the container.

D. magna was used as a bio-indicator in this bioassay and the toxicity of BPA was determined after different processes in Tu unit and LC_{50}. Tu rate was achieved from the below equation [37–40]:

According to the probit method, LC_{50} of BPA was determined in 24, 48, 72 and 96 h and the results were presented in Tables 2–6. This LC_{50} in the mentioned contact times were 42.94, 34.48, 24.26 and 20.22 mg/L on *D. magna*, respectively (Fig. 4). The LC_{50} of H_2O_2 processes in the mentioned contact times were 48.39, 38.55, 28.27 and 21.71 mg/L on *D. magna*, respectively (Fig. 5). LC_{50} of the product of US-35 kHz in the mentioned contact times were 50.75, 41.07, 32.06, and 22.48 mg/L on *D. magna*, respectively (Fig. 6). LC_{50} of the product of US-130 kHz in periods of 24, 48, 72 and 96 h were 53.81, 45.77, 38.53, and 24.40 mg/L on *D. magna*, respectively (Fig. 7). LC_{50} of the product of integrated method in periods of 24, 48, 72 and 96 h were 80.46, 69.02, 52.36, and 41.23 mg/L on *D. magna*, respectively (Fig. 8). Comparison of bioassay

Table 2
Data from toxicity testing on *D. magna* using synthetic samples before process (only BPA)

Parameter	Time (h)			
	24	48	72	96
LC 50 (mg/L) Fig. 4	94.42	48.34	26.24	22.20
LC 50 (mg/L) (95% confidence limit; upper bound)	26.65	62.62	90.29	67.25
LC 50 (mg/L) (95% confidence limit; lower bound)	88.26	74.11	95.18	17.15
Toxicity unit (TU)	33.2	9.2	12.4	94.4

Table 3
Data from toxicity testing on *D. magna* using synthetic samples using H_2O_2

Parameter	Time (h)			
	24	48	72	96
LC 50 (mg/L) Fig. 5	39.48	55.38	27.28	71.21
LC 50 (mg/L) (95% confidence limit; upper bound)	15.63	05.52	34.39	53.28
LC 50 (mg/L) (95% confidence limit; lower bound)	06.37	38.26	44.16	15
Toxicity unit (TU)	07.2	59.2	54.3	61.4

Table 4
Data from toxicity testing on *D. magna* using synthetic samples using US-35kHz process

Parameter	Time (h)			
	24	48	72	96
LC 50 (mg/L) Fig. 6	75.50	07.41	06.32	48.22
LC 50 (mg/L) (95% confidence limit; upper bound)	20.73	39.65	77.83	56.28
LC 50 (mg/L) (95% confidence limit; lower bound)	56.35	18.22	69.18	73.16
Toxicity unit (TU)	97.1	43.2	12.3	45.4

Table 5
Data from toxicity testing on *D. magna* using synthetic samples using US-130 kHz process

Parameter	Time (h)			
	24	48	72	96
LC 50 (mg/L) Fig. 7	81.53	77.45	53.38	4.24
LC 50 (mg/L) (95% confidence limit; upper bound)	53.80	92.73	61.57	68.30
LC 50 (mg/L) (95% confidence limit; lower bound)	24.37	67.26	08.23	57.18
Toxicity unit (TU)	86.1	18.2	59.2	09.4

Table 6
Data from toxicity testing on *D. magna* using synthetic samples using US/H_2O_2 process

Parameter	Time (h)			
	24	48	72	96
LC 50 (mg/L) Fig. 8	46.80	02.69	36.52	23.41
LC 50 (mg/L) (95% confidence limit; lpper bound)	68.115	87.97	53.67	15.53
LC 50 (mg/L) (95% confidence limit; lower bound)	87.63	89.53	25.41	25.31
Toxicity unit (TU)	24.1	45.1	91.1	42.2

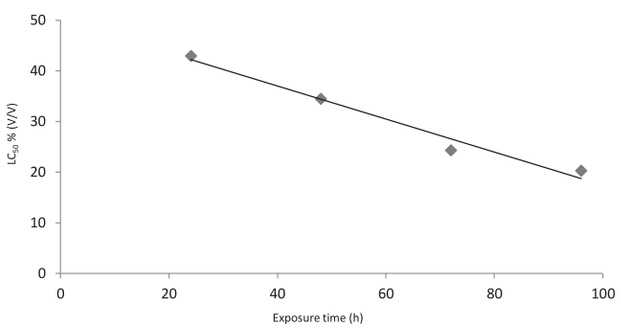

Fig. 4. LC_{50} from BPA toxicity on *D. magna* at different times.

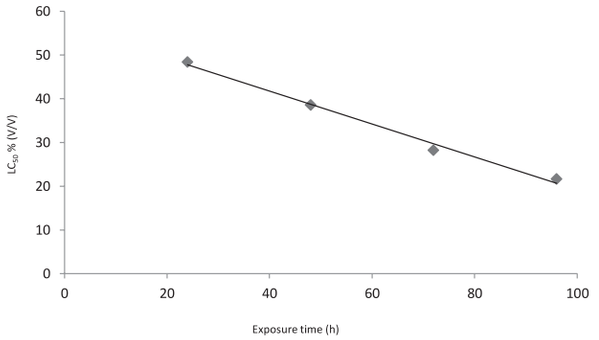

Fig. 5. LC_{50} from BPA toxicity in solution after H_2O_2 process at different times.

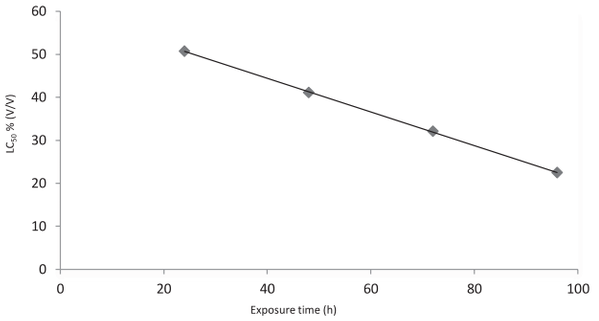

Fig. 6. LC_{50} from BPA toxicity in solution after US-35 kHz process at different times.

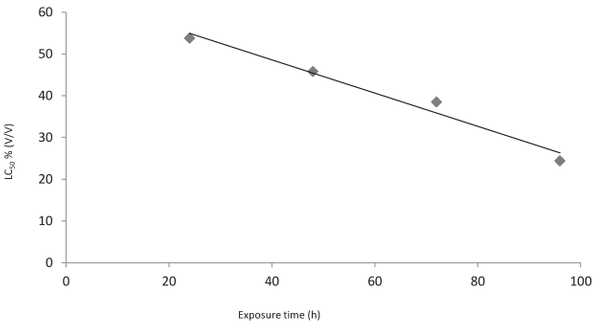

Fig. 7. LC_{50} from BPA toxicity in solution after US-130 kHz process at different times.

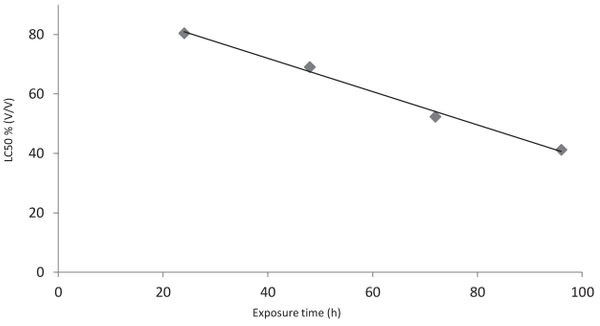

Fig. 8. LC_{50} from BPA toxicity in solution after US/H_2O_2 kHz process at different times.

results of BPA products shows that the toxicity of the initial solution (BPA with toxicity index of 2.33) was reduced in a 24 h period to 1.24, 1.86, 1.97, 2.07 respectively, after processed with US (130 and 35 kHz) and integrated methods.

$$Tu = 100\% / LC_{50}$$

The dye toxicity effect on *D. magn*a studied by Dehghani et al. The authors suggested that LC_{50} and TU are given for all time (24, 48, 72, and 96 h) are 124.9, 106, 111.6 and 91.55 mg/l, respectively. Corresponding values for TU are 0.8, 0.9, 0.94, and 1.1, respectively [25].

The LC_{50} Values at 24 and 48 h (mg/L) of 15 chemical compounds on *D. magna* investigated by Lucia et al (2000); paraoxon 0.00055 and 0.00019 – parathion 0.00219 and 0.00216 – DCA 0.271 and 0.100 648 – chlorpyrifos 0.344 and 145 – mercurous chloride 0.0027 and 0.002 – cadmium chloride 0.071 and 0.017 – copper sulfate 0.399 and 0.0826, zinc sulfate 35.403 and 4.029 – sodium dichromate 1.854 and 0.778 – chromous chloride 40.507 and 21.531 – sodium bromide 15,322 and 7451 – ethanol 9,788 and 5,680 – methanol 4,816 and 3,289 – SDS 45.898 and 19.129 – DBS 38.514 and 9.546, respectively. Also, acute toxicity of 54 chemical compounds to *D. magna* (24-h LC_{50} values) evaluated by Lucia et al (2000); acetic acid 10.79, amitriptyline 1.151, amphetamine sulfate 60.434, aniline 0.9, arsenic trioxide 7.5, aspirin 1,468.2, cadmium chloride 0.071, carbon tetrachloride 69.37, chloroform 64.23, chlorpyrifos 0.0037, chromous chloride 40.501, copper chloride 0.172, copper sulfate 0.399, DBS 38.514, DCA 0.271, diazepan 4.271, diazinon 0.0009, dichlorvos 0.00006, digitoxin 24.21, disulfoton 0.055, endosulfan 0.620, ethanol 9,788, ethylene glycol 4,8582, fenitrothion 0.0002, ferrous chloride 74.41, ferrous sulfate 14.28, formaldehyde 57, hexachlorophene 0.1982, isopropanol 6,850.26, lindan 14.5, malathion 0.354, mercurous chloride 0.0027, methanol 4,816, methyl parathion 0.00000031, paraoxon 0.00055, parathion 0.002189, p-chloroaniline 13, p-cresol 14, pentachlorophenol 0.44, phenobarbital 1,400.3, phenol 9.129, p-nitrophenol 11, quinine sulfate 44.8, SDS 45.898, sodium bromide 15,322, sodium chloride 1,022.6, sodium dichromate 1.854, sodium fluoride 307.7, stannous chloride 60.8, thallium sulfate 8.1, thiometon 5.49, toluene 8.0, zinc sulfate 35.403[41].

There were many studies done about the BPA toxicity effect on *D. magna*. For example one of them reported 10.5 mg/L LC_{50} of 24 and 48 h of BPA. Also another study determined 35.8 mg/L of 24 h LC_{50} of BPA [42,43].

4. Conclusion

Water and Wastewater containing BPA can be an important environmental pollution source and can cause serious hazard for human and environment. Sono chemical treatment is an effective technique in degradation of resistant pollutants in the environment. Moreover, using *D. magna* has a special place in bioassay tests today. Therefore, this study determines the toxicity of BPA before and after ultrasonic and hydrogen peroxide processes in separately and combined forms.

Totally related results to BPA toxicity are different. The cause of this difference is because of the physical, chemical and environmental factors effects on bio-indicator important role in bioassay tests. Thus, the difference might be too much. The range of toxicity in BPA after the mentioned processes is respectively: H_2O_2 > US-35kHz > US-130kHz > US/H_2O_2

Thus according to this study, it can be concluded that each of these methods had the potential of reducing toxicity of BPA and the theory of BPA removal was accepted and due to the process the toxicity of inlet and outlet of the reactors were presented. Comparison between two frequencies of US processes showed that products had higher toxicity after being composed with 35 kHz frequency than 130 kHz and as the result by-products would be more and toxicity of the effluent would be higher. But in the integrated method, results were much better and toxicity was reduced more than result of each method separately.

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