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Production of Hydroxyl Free Radical, the Main Mechanism for Removing Steroid Hormones by Ultrasound

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Abstract

Background: *One of the new methods for elimination or destruction of estrogen hormones is ultrasound irradiation. The aim of this study was to determine the main mechanism of steroid hormones removal by ultrasound.*

Methods: *In this study, estrogen (E1) and 17 beta-estradiol (E2) were irradiated with ultrasound at different frequencies, powers, and exposure times in two cases: with and without butyl alcohol in a batch-mode cylindrical reactor made of Plexiglas in the amount of one liter. Residual concentrations of hormones were measured by solid phase extraction and gas chromatography-mass chromatography (GC-MS).*

Results: *The result showed that ultrasound has high ability to remove hormones E1 and E2 (between 56.3% and 79.2%). Also, after adding butyl alcohol which is a free radical scavenger, the removal efficiency of ultrasound in both hormones was greatly reduced but didn't reach to zero, so the main mechanism of hormones removal was hydroxyl free radical production.*

Conclusions: *Due to the high efficiency of ultrasound for the removal as well as defects in other removal methods, more studies about optimization of the effective parameters on it, and technical and economical comparison with other removal methods are needed.*

Keywords: Steroid Hormones, Ultrasound, Removal mechanism, Hydroxyl Free Radical.

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Introduction

Steroid hormones are one of the major pollutants in water resources because they can cause fast death to fish and other aquatic organisms; moreover, they also increase the risk of some cancers, such as breast cancer.[1,2] These hormones mostly are produced in human and animal bodies and then enter into the environment, but they also exist in some artificial chemical compounds such as shampoo and cosmetics.[3] In recent years and after determining the risks of hormones for organisms, some researcher worked on the measurements of levels of these compounds in water resources and other environments, and also the methods to remove them. The following methods are some of the ways studied to remove hormones: biological methods such as activated sludge and oxidation ditch[4]; and advanced oxidation processes such as peroxon; and physiochemical methods such as activated carbon adsorption.[5] One of the new methods for elimination or destruction of hormones is ultrasound, which has high removal efficiency and does not produce dangerous by-products for health, and meanwhile requires low electricity.[6] These waves, first discovered by Francis Galton in 1876, are produced by two methods: Piezoelectric (strain interaction between mechanical pressure and electrical power); and Magnetostriction (creation of ultrasonic waves in the electromagnetic field). This method is used for curing of new injuries, restoring skin elasticity, removal of chemical organic pollutants from liquids, and curing old and chronic arthritis.[7] According to previous studies[8,9], the effects of power, frequency and ultrasound exposure time on removing steroid hormones have been investigated in previous studies, however, no study has been conducted to work on investigation of the main mechanism of hormones elimination by ultrasound waves. In ultrasound, we are faced with two mechanisms: cavitation and hydroxyl free radical production.[10] Ultrasound causes expansion and contraction in molecules and creates cavitation (molecular dissolution and generation of hot bubbles with high temperature [about several thousand Kelvin] and high pressure [about several hundred atmospheres])[11], also, it causes thermal decomposition of molecules to hydrogen atoms and hydroxyl free radicals [12], which have chemical reactions with organic materials known as sonochemical reactions. So, some researchers introduce cavitation phenomenon and production of hot bubbles as a main reason for removing materials by ultrasound, and some other researchers declare production of hydroxyl free radicals is the main reason.[13] Hydroxyl radicals are molecules with unpaired electrons and have high oxidizing properties and, as a result, can eliminate various molecules in every environment.[14] Also, ultrasound can form many hot spots, where the temperature inside reaches up to 5,000 °C. These spots quickly disappear after formation and they remove molecules attached to them or molecules that have been confined to them.[15] With regard to the high ability of ultrasound for removing steroid hormones and generating little by-products, researchers are looking for ways to increase the efficiency of this method. For this purpose, first we must know the main mechanism of hormones removal by this method and then define the effective factors on the mechanism to improve the efficiency of this method by optimizing these factors. So the aim of this study was to determine the main mechanism of steroid hormones removal by ultrasound.

Materials and Methods

As mentioned previously, ultrasound likely eliminates organic materials with two mechanisms: production hydroxyl radicals and creation of cavitation (hot spot). In order to determine that hormones removal by ultrasound is due to oxidation by free radicals, or is caused by cavitation and hot bubbles, a free radicals scavenger is added to the environment, like N-butyl alcohol. N-butyl alcohol is a strong absorber of hydroxyl radicals. If the ultrasound process leads to the production of hydroxyl radicals, with ingression N-butyl alcohol in the reactor, hydroxyl ions absorption is done by hydroxyl radicals and, as a result, removal of hormones decreases. On the other hand, if no reduction occurs in removal efficiency by adding N-butyl alcohol to the reactor, oxidation assumption through created free radicals by the effect of giving ultrasound becomes void.

All required chemicals, solvents, and hormones were bought from the company Sigma-Aldrich, UK and had high purity (greater than 97%). In this study, estrogen (E1) with a purity of 100% and 17 beta-estradiol (E2) with purity of 97.1%, were studied. The reason for choosing these two hormones was their presence in wastewater at higher concentrations than other hormones, as well as differences in their chemical structure, molecular weight, and properties. Since the concentration of these hormones in municipal wastewater in previous studies was estimated between 485 and 535 ng per liter, so the concentration of 550 ng per liter was used in this study. Methanol was of HPLC grade. Also, for extraction of solid phase, cartridges of 3 ml per 500 mg Varian bond by the Varian Company were used.

In this study, a cylindrical reactor made of Plexiglas in the amount of 1 liter for batch reactor was used (Figure. 1). The reactor contents were stirred by a stirrer magnet with low speed. The source of ultrasound generation was the device Model UGMA-5000 with three transducers, 30, 45, and 60 KHz, equipped with a titanium probe with a diameter of 20 mm. The input power of the device was adjustable from 60 to 120 watts.

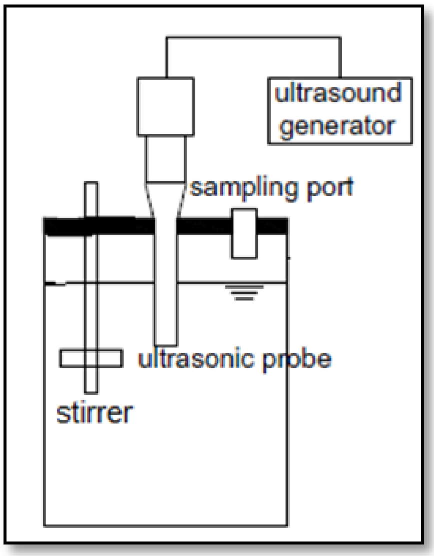

Figure 1: Applied reactor

Since the pH of municipal wastewater is at neutral level, in this study constant pH (7) was used and the effect of ultrasound power (70 and 110 watt), frequency (30 and 60 KHz), and exposure time (30, 60, 90, and 120 min) on the removal of E1 and E2 in two cases, with presence of 5 ml of N-butyl alcohol and without presence of 5 ml N-butyl alcohol, was investigated. These powers, frequencies and times previously had a significant effect on the efficiency of ultrasound. The concentration of hormones was 550 ng per ml and each test was repeated three times.

In this study, the effects of frequency, power, E1 and E2 concentrations and exposure time in two cases, with presence of 5 ml of N-butyl alcohol and without presence of 5 ml N-butyl alcohol, on removal efficiency of the hormones was investigated. Data analysis was performed using SPSS version 19. Independent samples t-test was used for evaluation of mean differences in hormones removal in various frequencies and powers, and one-way analysis of variance (ANOVA) was used for evaluation of mean differences in hormones removal in various exposures times. Factorial ANOVA was used for evaluation of mean differences in hormones removal in two cases with presence of N-butyl alcohol and without presence of N-butyl alcohol. Also, Tukey HSD was used to determine the contribution of each parameter, power, frequency and ultrasound time exposure on the removal of hormones. Mean differences at the level of 0.05 were considered significant.

Residual concentrations of hormones were measured by solid phase extraction and gas chromatography-mass chromatography (GC-MS).[16] In this method, cartridges of 3 ml per 500 mg Varian bond by Varian Company and methanol solvent, were used. Then, drying operations in Genevac EZ-2 evaporator and extraction operations by bistrifluoroacetamide were done, and finally, hormone levels and GC-MS analyses conducted by the Agilent 6890N device, were determined.

Results

Tables 1 and 2 show the results of removing E1 and E2 without N-butyl alcohol. The results showed that ultrasound has high ability in removing hormones E1 and E2 (between 56.3% and 79.2%). Also, the levels of removal for both hormones were almost alike.

Table 1: Removal rate (%) of estrogen by ultrasound in pH=7 without N-butyl alcohol

Power (Watt)	Frequency (KHz)	Time (min) 30	60	90	120
70	30	56.3±0.41	56.7±0.32	57.3 ±0.22	61.2 ±0.14
	60	61.5±0.21	63.1 ±0.54	65.6 ±0.16	68.9 ±0.42
110	30	69.1±0.16	70.5 ±0.43	70.8 ±0.51	72.3 ±0.17
	60	76±0.11	76.1 ±0.21	77.8 ±0.33	79.2 ±0.33

Table 2: Removal rate (%) of 17 beta- estradiol by ultrasound in pH=7 without N-butyl alcohol

Power (Watt)	Frequency (KHz)	Time (min) 30	60	90	120
70	30	55.8±0.15	56.4±0.32	58.8±0.41	60.8±0.62
	60	60.8±0.17	62.8±0.15	64.2±0.15	67.5±0.34
110	30	68.6±0.16	69.8±0.14	70.5±0.12	71.8±0.23
	60	73±0.14	75.6±0.16	76.2±0.14	78.2±0.24

Tables 3 and 4 show the results of hormones removal with N-butyl alcohol. According to this, by adding N-butyl alcohol the removal efficiency was greatly reduced in all frequencies, powers, and exposure times.

Table 3: Removal rate (%) of estrogen by ultrasound in pH=7 with N-butyl alcohol

Power (Watt)	Frequency (KHz)	Time (min) 30	60	90	120
70	30	11.4 ±0.21	11.8 ±0.35	12.5±0.62	16.4 ±0.13
	60	16.5±0.25	18.2 ±0.21	19.9±0.13	22.8±0.32
110	30	24.3±0.26	25.3±0.31	25.9±0.52	27.3±0.14
	60	28±0.12	29.5±0.24	29.9±0.32	30.8 ±0.31

Table 4: Removal rate (%) of 17 beta estradiol by ultrasound in pH=7 with N-butyl alcohol

Power (Watt)	Frequency (KHz)	Time (min) 30	60	90	120
70	30	11.6±0.14	11.9±0.49	12±0.42	16.9±0.29
	60	16.1±0.13	18.9±0.12	20.3±0.12	23±0.23
110	30	23.9±0.11	26.1±0.11	26.4±0.14	26.9±0.27
	60	27.8±0.23	28.7±0.12	29.2±0.12	30.1±0.21

As tables 1 and 2 show, ultrasound has high ability for removing hormones E1 and E2 (between 56.3% and 79.2%). Also, the levels of removal for both hormones were almost alike. High similarities of molecular structure of these two hormones can be the main reason for these same levels. According to the results, with increasing power, frequency and exposure time, removal efficiency of these two hormones increased (Figures. 2 and 3), but: a) Independent samples t-test statistical analysis showed there is significant difference between concentrations of E1 and E2 in reactor influent and effluent at different frequencies (P for E1 and E2 were 0.006 and 0.004, respectively) and at different powers (P for E1 and E2 were 0.009 and 0.008, respectively). b) Statistical analysis of one-way analysis of variance (ANOVA) showed there is no significant difference between concentrations of E1 and E2 in reactor influent and effluent at different exposure times (P for E1 and E2 were 0.14 and 0.18, respectively).

Discussion

In fact, the ultrasound waves were so energetic that they acted in a short time, and therefore, increased exposure time caused no significant increase in removal levels. But increasing the frequency and powers led to increasing removal efficiency because they increased levels of ultrasound energy and production of hydroxyl radicals. Results of the studies of Liu et al,[8] Saleh et al.[9] and Wang et al.[17] correspond with our study. Liu et al. showed that increasing exposure time to ultrasound had no significant increase on removal efficiency of organic material, as removal efficiency of the activated carbon impurities from 23% at 30 min reached to 31% at 90 min. They defined 60 min as an optimum exposure time in their study.[15] Saleh et al. showed in a study on chromogenic acid that increasing frequency leads to improvement of removal efficiency of organic acids by ultrasound, as the removal levels from 56% at the frequency of 45 kHz reached to 82% at the frequency of 60 kHz. They also showed that by increasing the frequency, the energy of ultrasound increases and as a result the production of hydroxyl radicals and also the internal temperature of cavitation bubbles was raised.[16] The study of Wang et al. on the enzymatic degradation by ultrasound revealed that power increase through improving the energy of cavitation bubbles increases the enzymes removal, as the removal level from 46% at the power of 70 Watt reached to 78% at the power of 85 Watt. They also showed that power and frequency are effective parameters on the removal of organic materials by ultrasound waves.[17]

As Tables 3 and 4 show, the removal efficiency greatly reduced in all frequencies, powers, and exposure times by adding N-butyl alcohol. Since the N-butyl alcohol is a free radicals scavenger and by entering into an environment it reacts with free radicals, so the reduction of removal levels after entering N-butyl alcohol is due to the reduced number of free radicals. Therefore, it can be concluded that the main mechanism of hormones removal by ultrasound is production of hydroxyl radical, but other mechanisms such as cavitation are effective, and for this reason, despite the elimination of free radicals, hormones removal occurs again. In order to ensure that all free radicals are absorbed by the N-butyl alcohol, its addition is continued until the formation of detectable residue.

The result of statistical analysis of Factorial ANOVA showed that the hormone removal for both hormones E1 and E2 after adding N-butyl alcohol increased significantly over hormone removal without adding N-butyl alcohol (P = 0.003). Also, the Tukey statistical analysis showed a significant difference between E1 and E2 hormone removal after

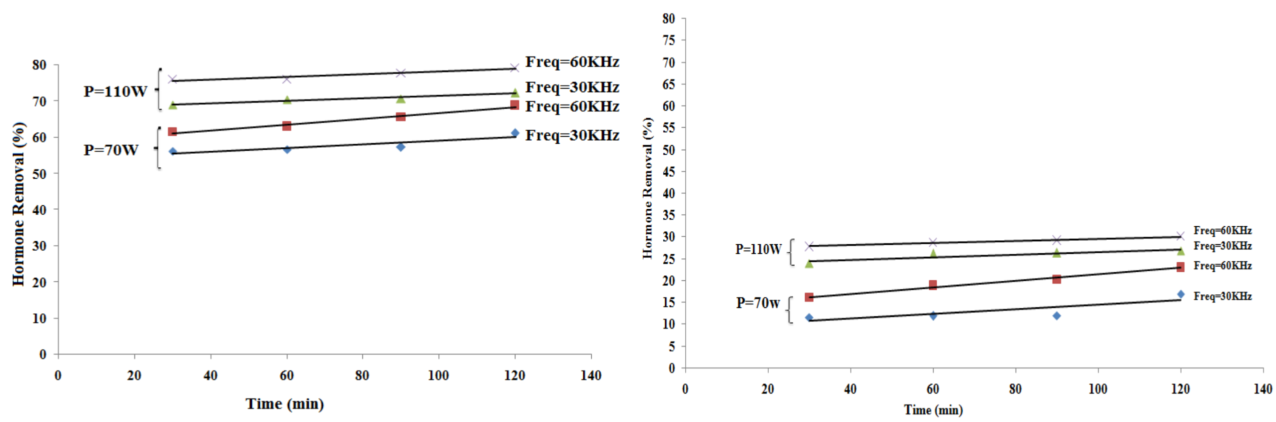

Figure 2: Comparison of E1 hormone removal by ultrasound with and without N-butyl alcohol. (Left: without, right: with n-butyl alcohol)

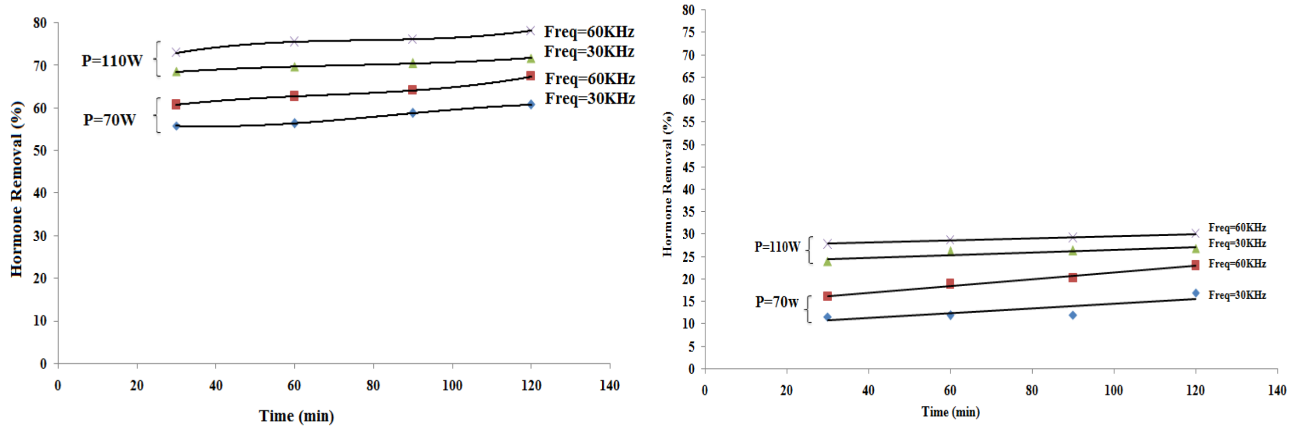

Figure 3: Comparison of E2 hormone removal by ultrasound with and without N-butyl alcohol. (Left: without, right: with n-butyl alcohol)

adding N-butyl alcohol at all frequencies and powers (P = 0.002), but the difference between the exposure times was not significant (P = 0.19).

The results of our study correspond with results of Rehman et al.[18], Zhang et al.[19], Xu et al.[20], Chu et al.[21], Zhou et al.[22] and Mahvi et al.[23].

Rehman et al. examined the production of free radicals in three cold plasma, ultrasound and ionic radiation. In part of their study, they conclude that ultrasound methods are more capable in production of hydroxyl radical than two other methods. Also, the amount of hydroxyl radical production greatly increases in frequencies higher than 45 kHz and powers more than 90 watt.[18] Zhang et al. investigated the production of free radicals resulting from ultrasound in red wine and concluded hydroxyl free radicals are produced during ultrasound irradiation as one of the intermediate products.[19] In a study by Xu et al, they claimed decomposition of protoporphyrin by ultrasound came from hydroxyl free radicals and concluded that cavitation mechanism has a low role in the elimination of the drug and spontaneous drug decomposition has the lowest effect.[20] Also, the study by Chu et al. on amylum polymerization and butyl acrylate revealed that, by ultrasound, a large number of hydroxyl radicals are created which accelerates the polymerization process. In this study, the role of hot bubbles in the polymerization was very low.[21] In the study by Zhou et al. on hexavalent chromium by nanoparticles of iron/nickel in the vicinity of the ultrasound, they indicated that with increasing frequency the production of hydroxyl radical increases and consequently, the removal efficiency of chromium increases.[22] The study by Mahvi et al. on leachate showed that the maximum removal level of COD by ultrasound in pH = 7 and power = 70 watt was 37% and, after adding 5 ml N butyl alcohol, the level was reduced to 19.9%. So adding N-butyl alcohol reduced the amount of hydroxyl ions in the environment, hence the removal level of organic materials decreased.[23]

In this study the removal mechanism of steroid hormones by ultrasound was investigated. For this purpose, estrogen and 17 beta-estradiol were irradiated with ultrasound waves at different frequencies, powers, and exposure times in two cases: with and without butyl alcohol. The results showed that ultrasound has high ability in removing hormones E1 and E2 (between 56.3% and 79.2%). Also, after adding butyl alcohol which is a free radical scavenger, the removal efficiency of both hormones greatly reduced but didn't reach to zero, so the main reason for hormones removal is hydroxyl free radical. However, the cavitation phenomenon plays a role in the removal. Therefore, due to the high efficiency of ultrasound for the removal, as well as defects in other methods of removal, it is suggested that researchers study optimizing the effective parameters on ultrasound and technical and economical comparison with other methods of removal.

Conflict of Interest

The authors declared that they have no conflict of interest.

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