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Escherichia coli' nin Ultraviyole ve Ultrases Enerjisi ile Dezenfeksiyonu

The Disinfection of *Escherichia coli* by Ultraviolet Intensity and Ultrasound Power

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Öz: Organik bileşenler ve mikroorganizmalar gibi fiziksel, kimyasal ve biyolojik bileşenler fabrikalardan, evlerden, tesislerden ve diğer bazı kaynaklardan atık su ile birlikte salıverilirler. Mikrobiyal kirlilikler arasında bakteriler, virüsler ve funguslar sayılabilir. Bu çalışmada, atık sulardan *Escherichia coli* O157:H7 suşunun ultrases enerjisi ve ultraviyole ışınları kullanılarak dezenfeksiyonu amaçlanmıştır. Parametre olarak, ultrases enerjisinin %60'lık amplitüdü ve 254 nm'de 88 W/m^2 ışık şiddeti kullanılmıştır. Ultrases kaynağı olarak ultrasonic jeneratör ve ultraviyole ışık kaynağı olarak da Pen-Ray ultraviyole lamba kullanılmıştır. Denemeler sırasında sıcaklık 37°C' de ve TiO_2 miktarı 300 mg' da sabit tutulmuştur. Ultrases enerjisinin etkisini incelemek için, ultrases enerjisi sistemde tek başına kullanılmıştır. Aynı zamanda, ultraviyole ışığın etkisini incelemek için, ultraviyole ışık sistemde tek başına kullanılmıştır. Sinerjistik etkinin gözlenmesi için ise ultrases ve ultraviyole ışığın her ikisi sistemde birlikte kullanılmıştır. Deney sonuçlarından, bakterinin tamamen yok edilmesi ultraviyole ışığın (UV) yalnız kullanıldığı sistemde 30. dakikadan sonra, ultrases enerjisinin (US) tek başına kullanıldığı denemelerde 40. dakikadan sonra ve ultrases ve ultraviyole ışığın her ikisinin birlikte eşzamanlı kullanıldığı sistemde ise 8. dakikadan sonra olduğu görülmüştür. En fazla bakteri giderimi ultrases ve ultraviyole ışığın eşzamanlı olarak birlikte kullanıldıkları sistemde olduğu tespit edilmiştir. Sesin ve ışık enerjilerinin birlikte kullanımı bakteri giderimi için daha fazla $^{\bullet}OH$ radikalinin üretilmesini sağladığından en etkili proses olmuştur. Ayrıca, 254 nm dalga boyunun daha fazla delici ve tahrip edici özellikte olmasından dolayı, bu çalışmada daha etkili bir dezenfeksiyon gözlenmiştir.

Anahtar Kelimeler — Ultrases, ultraviyole ışık, *Escherichia coli*, su kirliliği.

Abstract: Physical, chemical and biological constituents such as organic compounds and microorganisms are released by wastewaters from fabrics, homes, facilities and other resources. Bacteria, viruses and fungi can be described as microbial pollutants. In this study the disinfection of Escherichia coli O157:H7 strain from the wastewater was aimed by using ultrasound power and ultraviolet light intensity. The 60% amplitude of ultrasound energy and also, 88 W/m2 light intensity at 254 nm wavelength was used. The ultrasound source was an ultrasonic generator and the ultraviolet light source was Pen-Ray ultraviolet lamp. The temperature was constant at 37°C temperature and the amount of TiO2 was 300 mg during the experiments. For investigating the effect of ultrasound energy, it was used alone to the system. Also, for the effect of ultraviolet light, it was used alone to the system. For the synergistic effect of ultrasound and ultraviolet, they were used together to the system. The results showed that the completely disappearance was seen at 30 min. when the ultraviolet light (UV) was used alone, 40 min. when the ultrasound (US) was used alone and 8 min when the ultrasound and the ultraviolet light were used simultaneously. The most disinfection was determined when the ultrasound and the ultraviolet light were used simultaneously. Together using of the sound and light energies proved to be the most effective process on bacterial disinfection by generating greater •OH radicals. Also, having more piercing and devastating properties of 254 nm wavelength, it was seen more effective disinfection in this study.

Keywords — Ultrasound, ultraviolet light, Escherichia coli, water pollution

1.Introduction

There are several types of pollutant in wastewaters. The release of treated and untreated effluent to water-bodies is a global occurrence and can be a significant source of pathogenic microorganisms (Barrett *et al.* 2016). Microbial pollutants such as bacteria, viruses, fungi are one of the significant harmful sources of wastewaters. They have damaged health of livings. It can be pronounced that the *Escherichia coli* is the most important contamination markers among the bacteria for the health. *E. coli* is normally present in the gastrointestinal systems of people and animals as a part of the natural microflora. Currently, and largely as a result of *Escherichia coli* O157:H7,producing *E. coli*, outbreaks associated with lettuce, baby spinach, other tender greens, and leafy culinary herbs, many growers and fresh processors have adopted preharvest testing and/or a finished goods 'test to release' approach before accepting a field lot for harvest or transporting product to processing operations or commercial distribution channels following a negative finding of target pathogens (D'Lima and Suslow, 2009; Velasco *et al.,* 2015). *E. coli* is a member of *Enterobacteriaceae*, is a fecal coliform. These bacteria are the most widely adopted indicator of fecal pollution in food and water (Tavakoli *et al.,* 2008). The existence of these microorganisms in fresh water arises from the release of wastewater to the natural water sources. Accordingly, various methods have been developed for eliminating the microorganisms from wastewaters before they reach the natural water sources. The physical methods are commonly used for removing the pollutants. However, these methods are not destructive enough and they forward the organic compounds to another phase. The biological processes such as slow sand filters and biologically active carbon are expensive and difficult applications. Some traditional disinfection methods such as chlorine based technologies lead to the formation of undesirable chloro-organic disinfection by-products like trihalomethanes and haloacetic acids with carcinogenic and mutagenic effects on mammals (Rook, 1974, Nissinen *et al.,* 2002). Accordingly, the use of alternative disinfection systems should be evaluated as possible alternative to these methods.

In recent years, there is great effort to find suitable technologies for treatment of wastewaters (Casani *et al.*, 2005; Artés *et al.*, 2009; Olmez and Kretzschmar, 2009; Anese *et al.,* 2015) and also, some new methods have gained great attention for disinfection of wastewaters. Power ultrasound has been suggested as an alternative technology for wastewater decontamination (Neis and Blume, 2002; Piyasena *et al.*, 2003; Anese *et al.*, 2015). Ultrasound frequencies higher than 20 kHz are actually considered safe, non-toxic and environmentally friendly (Kentish and Ashokkumar, 2011; Anese *et al.*, 2015). During ultrasound treatment cavitation phenomena occur into the liquid medium causing a rapidly alternating compression and decompression zones, which are in turn responsible for generating shock waves with associated local very high temperatures and pressures, as well as free radicals and hydrogen peroxide (Leighton, 1994; Mason *et al.*, 2003; Anese *et al.*, 2015). Improved efficiency of ultrasound technology can be obtained by its combination with ultraviolet irradiation. In UV mechanism, upon irradiation, valence band electrons are promoted to the conduction band leaving a hole behind (Eq. (I)). The hydroxyl radicals can be produced as (Daneshvar *et al.*, 2004; Konstantinou and Albanis, 2004; Behnajanady *et al.*, 2006; Saygi and Tekin, 2013)

$$TiO_2 + h\nu \longrightarrow e^- + h^+ \qquad (I)$$
$$e^- + h^+ \longrightarrow heat \qquad (II)$$
$$h^+ + H_2O \longrightarrow {}^{\bullet}OH + H^+ \qquad (III)$$
$$h^+ + OH^- \longrightarrow {}^{\bullet}OH \qquad (IV)$$

In US mechanism, the hydroxyl radicals can be produced by the following equations (Wu, 2008; Ertugay and Acar, 2014; Monteagudo *et al*., 2014). When water is irradiated with US, $^{\bullet}OH$ radicals are formed by thermolysis of H_2O in the collapsing bubble (Eq. (V)).

$$H_2O \longrightarrow {}^{\bullet}H + {}^{\bullet}OH, \qquad (V)$$
$${}^{\bullet}H + {}^{\bullet}OH \longrightarrow H_2O, \qquad (VI)$$
$${}^{\bullet}H + O_2 \longrightarrow {}^{\bullet}HO_2, \qquad (VII)$$
$$2\,{}^{\bullet}HO \longrightarrow H_2O_2, \qquad (VIII)$$
$$2\,{}^{\bullet}HO_2 \longrightarrow H_2O_2 + O_2, \qquad (IX)$$
$${}^{\bullet}H + H_2O_2 \longrightarrow {}^{\bullet}OH + H_2O. \qquad (X)$$

Free radicals generated by the hemolysis of water have important role in destroying bacteria (Crum *et al*., 1999; Ince *et al*., 2001; Tezcanli *et al*., 2004; Anese *et al*., 2015). In recent years, the simultaneous use of ultrasound and ultraviolet light, *i.e*. the so-called UV+US has been studied regarding the efficiency of microbial disinfection. Simultaneous uses of them proved to be more effective than their individual usages (Mrowetz *et al*., 2003; Sahu and Parida, 2012; Yetim and Tekin, 2012).

In this study, advanced bacterial disinfection was aimed by generating more free radicals using ultrasound and ultraviolet light methods simultaneously. On this purpose, *E.coli* was exposed to UV, US and UV+US processes in order to appoint the most effective methods.

2. *Materials & Methods*

Experiments were performed in a Pyrex glass reactor. US was carried out by an ultrasonic generator (Cole Parmer, Ultrasonic homogenizer, 750 W, 20 kHz) with a cup horn probe. Pen-Ray UV lamp (Cole Parmer, 365 nm) was used as the radiation source in UV. The water was circulated continuously within the water jacket reactor by the constant temperature water circulator to keep the temperature stabile. The required O_2 for system was provided by a vacuum pump.

The commercial TiO_2 supplied by Degussa (P25) was used as photocatalyst. According to the manufacturer's specifications, P25 has an elementary particle size of 30 nm. a BET specific surface area of 50 m^2/g and its crystalline mode was 80 % anatase and 20 % rutile.

Escherichia coli O157:H7 strain was used throughout the study. The strain was grown aerobically in 250 mL flasks containing 100 mL of nutrient broth at 37 °C on a rotary shaker overnight. The final bacterial count was adjusted to 10^8 cfu/mL in bacterial suspension for further use. The cell viability of bacteria was tested on the Plate Count Agar (PCA). During the course of reactions, samples were taken from suspension at regular intervals for 60 min in triplicates. The viable count was performed on PCA plates after serial dilutions of the sample in phosphate-buffered solutions. All plates were incubated at 37 °C for 24-48-72 h.

Amplitude of ultrasound energy of 60 % (55.03 W), 254 nm wavelength, 88 W/m^2 light intensity and 37 °C temperature were used as the constant parameters for the experiments. The reactor was isolated from the outside light. Bacterial solution and 300 mg TiO_2 were introduced to the reactor. Experiments were carried out by continuously stirring with the magnetic stirrer. 5 ml samples of suspension were withdrawn at regular intervals. Samples were diluted nine times and each dilution was spread on PCA. US, UV, UV+US experiments were studied for displaying the most effective process on disinfection.

3. *Results & Discussion*

Bacterial disinfection data of the US, UV and UV+US at 88 W/m^2 light intensity and 254 nm wavelength was graphed in Fig.1. After 6 min the disinfection values were approximately $1.4x10^4$, $2.7x10^7$ and $8x10^0$ for UV, US, and UV+US processes, respectively. The complete disappearance was seen after 30 min for UV, 40 min for US and 8 min for UV+US.

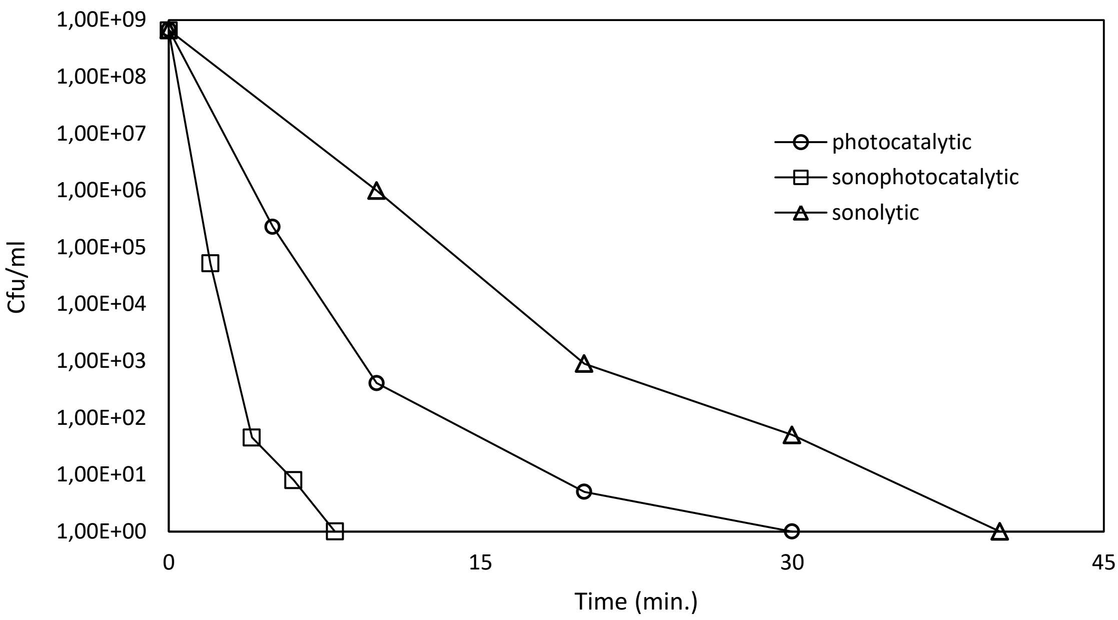

***Figure 1.** The disinfection of E. coli in US, UV and UV+US processes at 254 nm wavelength and 88 W/m^2 light intensity.*

The highest disinfection rate was obtained by the UV+US process. As seen from the Fig. 1, much more disinfection can be determined at UV and UV+US processes than US process. This can be attributed to the amount of light intensity. Increasing the light intensity the excited electron amount increases, and also, the occurring $^{\bullet}$OH radicals amount increase. Also, it can be attributed to the shortness of the wavelength too. It is a general knowledge that the frequency increases by the decreasing of the wavelength value. Therefore, the number of the wave passed in a second will increase. Thus, the number of waves effected to the bacteria increase. It is known that as the wavelength decrease, the piercing and the devastating properties increase. So that at 254 nm and at 88 W/m^2 light intensity more bacteria can be disinfected. Besides that, in US process bubbles collapse strongly on the catalytic surface. The collapsing of bubbles occurred by US at high temperatures and pressures cause the formation of $^{\bullet}$OH radicals. Hence, more holes and pores are formed. The surface area of the catalyst can also increase by these holes and pores. Also, catalyst surfaces can be cleaner by US for forming more $^{\bullet}$OH radicals. However, the wavelength and the light intensity are more effective than the collapse of bubbles on the disinfection. When these reasons are considered at the same time the synergistic effect of them would be strongest on the disinfection. So that the most disinfection is seen in UV+US process.

4. Conclusion

In this study, *Escherichia coli* O157:H7 strain was used for investigating the disinfection of bacteria by comparing the US, UV and UV+US processes. The obtained results from this study can be summarized as follows:

- At 60 % (55.03 W) amplitude of ultrasound energy, 254 nm wavelength and 88 W/m^2 light intensity the most effective process was found to be UV+US on the disinfection due to generating more $^{\bullet}OH$ radicals in this process.
- In US process the collapsing of bubbles high temperatures and pressures occur and cause the formation of $^{\bullet}OH$ radicals.
- At 88 W/m^2 and the short wavelength as 254 nm, UV and UV+US processes are more effective than US process on the disinfection, due to irradiating more photons to the medium for generating much more $^{\bullet}OH$ radicals.

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