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Preliminary investigation on the optimum ultrasound frequency for the degradation of TNT, RDX, and HMX

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Abstract

The wastewater of the munitions industry varies in terms of the recalcitrant compounds depending on the products and the manufacturing process. The most commonly encountered energetic nitro-aromatic compounds in ammunition wastewater are trinitrotoluene (TNT), nitro-heterocyclic hexahydro-trinitrotriazine (RDX), and cyclotetramethylene-tetranitramine (HMX). These compounds are known to be very toxic and resistant to biodegradation. The aim of this study was to determine the appropriate ultrasound frequency to assist the anaerobic biodegradation of TNT, RDX, and HMX Therefore, the optimum ultrasound frequencies were investigated to degrade TNT, RDX, and HMX without destroying anaerobic microorganisms. The results showed that ultrasound irradiation was effective for the degradation of all the explosive compounds used. However, the maximum removal efficiencies and minimum microorganism lysis were achieved by the frequencies of 20 and 800 kHz.

Keywords: TNT, RDX, HMX, Ultrasound, Frequency

1. Introduction

Sound waves with a frequency of more than 20 kHz are called 'ultrasound'. The use of ultrasound in liquids causes periodical compression and thinning (dilution). The bubbles formed by this action grow and deflate within a few micro-seconds, a process called 'cavitation' (Mason 1991; Leong *et al.;* 2011). Ultrasonic cavitation creates a number of simultaneous mechanical, acoustical, and chemical changes in a liquid medium (Dehghani *et al.;* 2007; Sabari, 2010). Large cavitation bubbles occur at low frequencies (Aydin and Civelekoğlu, 2010) and exert huge hydro-mechanical shear forces. During the deflation of cavitation bubbles, the bubble pressure and adiabatic temperature rise dramatically (Patil and Pandit, 2007). Therefore, each bubble in the liquid can be considered as a micro-reactor. Due to the high temperature and pressure inside the bubble, the sonochemical process covers several pyrolytic and radical reactions inside the bubble and/or at the bubble-liquid interface (Zhang *et al.,* 2006). The extreme conditions created by cavitation leads to the thermal destruction of pollutants and generation of reactive hydroxyl radicals (Mason, 1991). Therefore, by pyrolytic processes, volatile pollutants are degraded inside the cavitation bubbles while non-volatile pollutants are degraded by the hydroxyl radical in the bulk liquid (Tiehm, 1999; González-García *et al.,* 2010).

In the literature, studies on the oxidation of environmental pollutants using ultrasound were carried out at frequencies between 20 kHz and 1000 kHz. However, in a hybrid system involving ultrasound and biological treatment, one of the two strategies are applied: (i) The simultaneous use of ultrasound with biological treatment or (ii) ultrasonic pre-treatment followed by biological treatment (Liu *et al.,* 2005; Ariunbaatar *et al.,* 2014). In the first strategy, the microbial growth rate should be equal or higher than the inactivation rate of microorganisms caused by the ultrasound. Therefore, the parameters causing the inactivation of microorganisms in sonobioreactors must be considered during the design procedure of the reactor.

It is known that low-frequency cavitation bubbles are larger than the bubbles formed at higher frequencies (Aydin and Civelekoğlu, 2010). Therefore, bubble deflation at low frequencies such as 20 kHz creates powerful hydromechanical shear forces. Sonochemical effects occur within the frequency range of 200 - 1000 kHz (Aydın and Civelekoğlu, 2010). A review of the literature showed that low-frequency ultrasound applied only at very low intensities for a short period of time is likely to do less harm to the microorganisms (Liu *et al.,* 2005; Ariunbaatar *et al.,* 2014). The microorganisms can be exposed to ultrasound for longer periods only by raising the frequency or lowering the intensity.

The wastewater composition of the munitions industry varies in terms of the content of recalcitrant compounds depending on the products and the manufacturing process. In general, ammunition wastewater consists of 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX), 1,3,5,7-tetranitro-1,3,5,7-tetra-azo-ethane (HMX), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 1,3-dinitrobenzene (1,3-DNB), 1,3,5-trinitrobenzene (1,3,5-TNB) and nitrobenzene. Among

these energetic nitro-aromatic compounds, TNT, nitro-heterocyclic RDX, and HMX are most commonly seen in ammunition wastewater (Sullivan *et al.,* 1979; Ju and Parales, 2010). These compounds are known to be very toxic and resistant to biodegradation (Sullivan *et al.,* 1979; Ju and Parales, 2010). According to the Environmental Protection Agency, TNT and RDX are classified as possible carcinogen compounds (Group C). The recommended limits for the concentrations of TNT and RDX in aqueous media are 2 µg/L and 300 µg/L, respectively (Sullivan *et al.,* 1979). However, HMX is less toxic and has less mutagenic properties compared to TNT and RDX, and is not classified as a carcinogenic compound. The recently, anaerobic conversion of nitro-aromatics has become a topic of great interest, leading to an increase in the number of related research papers. Aromatic nitro groups are reduced to amines through a 6-electron transfer under anaerobic conditions (Razo-Flores *et al.,* 1997; Ghattas *et al.,* 2017). Reduction of nitro groups to nitroso derivatives, hydroxyl amines or amines can be achieved using a co-substrate with the addition of electron pairs and nitroreductase as a catalyst. Some studies have reported that most nitro-aromatics are degraded only under anaerobic conditions (Nishino and Spain, 2002; Zhang and Bennett, 2005; Ghattas *et al.,* 2017). However, very few species of anaerobes can convert nitro-aromatics to the end products of CO_2 and CH_4 (Razo-Flores *et al.,* 1997). Therefore, conversion of nitro-aromatics such as TNT, dinitrotoluene, 3,5-dinitrobenzoic acid, and 2-,3- and 4-nitrophenol to end products can only be achieved through a synergistic effect created by mixing cultures containing different bacteria (Hess *et al.,* 1990). In addition, HMX can be biodegraded in anaerobic medium by the addition of a co-substrate. The anaerobic degradation studies of HMX have revealed that it could not be oxidized only as a carbon source (Layton *et al.,* 1987).

Considering the studies in the literature, anaerobic biodegradation appears to be a promising method for the treatment of munitions wastewater. However, this process should be accelerated for industrial-scale implementations. Despite the availability of research focusing on the treatment of munitions wastewater via anaerobic biodegradation and ultrasound irradiation, to the best of our knowledge, there is no published study that investigated how to accelerate anaerobic biodegradation of the munitions pollutants using ultrasound. Hence, in this paper, we aimed to report on our preliminary studies on the determination of optimum ultrasound frequencies for the accelerating the anaerobic biodegradation of the compounds mentioned above.

2. Material and Method

2.1. Munitions solution

For the experiments to determine the optimum ultrasound frequency, the solutions were prepared in the laboratory. The common composition of wastewater was taken as a basis for the preparation of explosives; namely, 50, 35, and 5 mg/L for TNT, RDX, and HMX respectively. The explosives were dissolved in acetonitrile and then tap water was added to adjust the concentrations. The acetonitrile was then evaporated by heating. After the evaporation stage, a sufficient amount of glucose (500 mg/L) ($C_6H_{12}O_6.H_2O$) was added to the solution in order to observe the selective property of ultrasound. In each experiment, a total of 250 mL explosive solution was utilized.

The TNT, RDX, and HMX measurements were obtained by high performance liquid chromatography (Perkin Elmer) using a C18 column (5µm, 25 cm × 4.6 mm) at 25 °C. A mixture of 50% methanol and 50% distilled water was used as the carrier with a flow of 1 mL/min. The wavelength was determined to be 254 nm by an ultraviolet–visible detector (Keller *et al.,* 2011). For the determination of the glucose amount, the phenol-sulphuric acid method was used (Dubois *et al.,* 1956).

2.2. Mix Liquid Suspended Solid (MLSS)

To determine cell lysis due to sonication, MLSS analyses were utilised. For this purpose, known concentrations of MLSS were recorded at a wavelength of 550 nm and the calibration curve was obtained using the absorbance values (Farooq *et al.,* 2009).

A sample culture taken from the mesophilic anaerobic reactor acclimatized with explosives was diluted to an MLSS concentration of 1180±10 mg/L. Since this was a sensitive analysis, the tap water used for the dilution was stripped by nitrogen gas. For each experiment, only 250 mL of the diluted anaerobic mixed culture sample was used.

2.3. Ultrasound Generation & Transducers

The experimental system used in the study is depicted in Figure 1. Throughout the study, the frequencies of 20, 40, 60, 100, 200, 400, 600, and 800 kHz (MQ-20/40/60, MQ-100, MQ-200, MQ-400, MQ-600, and MQ-800), and a power of 400 W/L were applied. The surface area of the multi-frequency transducer (MQ-20/40/60) was 78.5 cm^2 and that of a single frequency transducer was 38.5 cm^2. A jacketed reactor with a 500 ml volume was fabricated using stainless steel and equipped with a Teflon lid with a hole of 1 cm in diameter for sampling. All the experiments were carried out at a constant temperature of 35±2 °C. Each experiment took 180 minutes. The ultrasonic power input was determined by calorimetric measurement (Mason, 1991; Petosic *et al.,* 2011), which was found equivalent to 75% of the electrical power input.

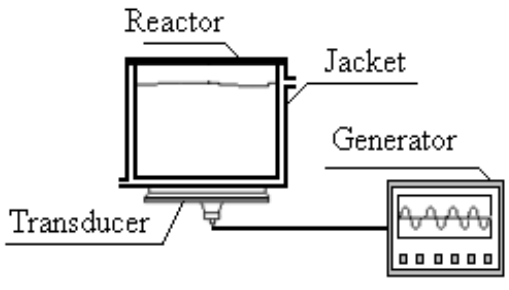


Figure 1. Schematic illustration of the experimental system

3. Results and Discussion

The aim of this study was to determine the optimum ultrasound frequencies for ultrasound-assisted anaerobic biodegradation of TNT, RDX, and HMX. The initial effect of ultrasound on the microorganisms was the reduction in flock size (Farooq *et al.,* 2009). As seen in Figure 2, within the first 20 minutes of the ultrasound application, the usual granular structure of the microorganisms present in an anaerobic digester was impaired; the MLSS concentration increased due to the reduction of the flock size with the increased turbidity resulting from the scattered microorganisms. Similar findings were obtained from different studies using pure cultures (Jacobs and Thornley, 1954) or mixed cultures of anaerobic microorganisms (Tiehm *et al.,* 2001). The effect of ultrasound on the microorganism flocks is regarded as a positive effect on the biodegradability of complex compounds since the interaction between the substrate and microorganisms becomes more efficient due to the enhanced cell permeability.

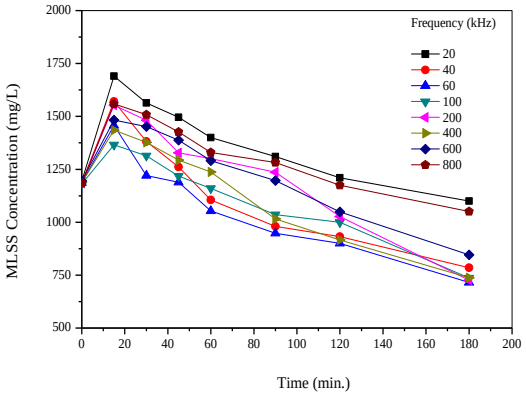


Figure 2. Change in the MLSS concentration by sonication time.

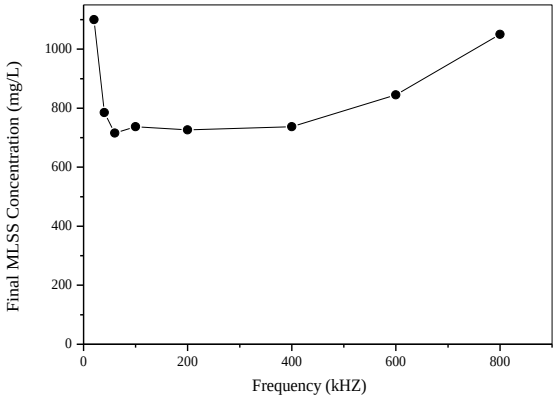


Figure 3. Final MLSS concentrations at different frequencies

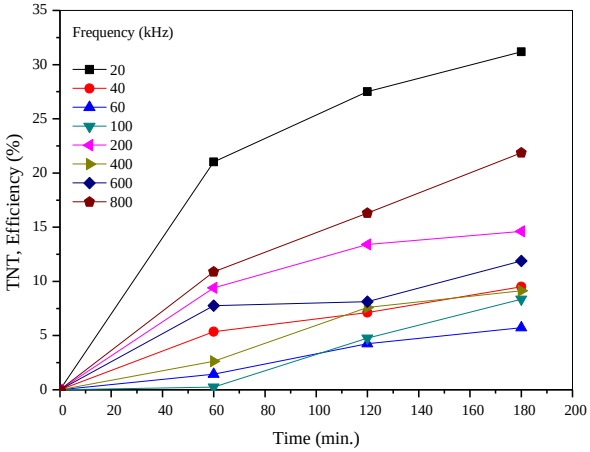
Furthermore, a secondary positive effect of ultrasound is the enhanced mixing at a macro scale, which increases the mass transfer operations in the reactor medium. However, as shown in Figure 2, after a certain time of exposure, the microorganisms started to disintegrate.

The cell walls were damaged as a result of the prolonged ultrasound exposure, which explains the decrease of the MLSS concentration. Hydro-mechanical shear forces and sonochemical reactions have been reported as the main causes for cell destruction (Neppiras, 1980; Le *et al.,* 2015). According to Liu *et al.,* (2005), hydro-mechanical shear forces produced by cavitation play a greater role in cell disintegration than sonochemical processes. On the other hand, Joyce *et al.,* (2002) argued that the formation of radicals during cavitation was another important factor affecting cell disintegration. Hydroxyl (OH•) radicals generated during ultrasound irradiation causes the physical disruption of the cell membranes, as pointed out by Motwani (2008). As a consequence, the cell wall is destructed and the oxidants entering the cell attack the internal vital structures. Hydrogen peroxide, one of the final products of sonication, has also been considered to be responsible for cell disintegration (Mason *et al.,* 2003). Finally, the increased regional temperature due to cavitation and bubble implosion has negative effects on the microorganisms.

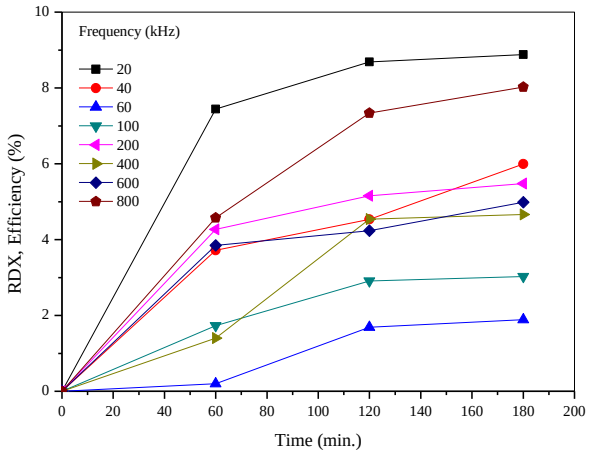
A number of researchers have also reported that ultrasound frequency has a crucial effect on the microorganism activity within the frequency range of 100 kHz to 1 MHz (Mason, 2010).

Figure 3 presents the MLSS concentration as a function of ultrasound frequency at the end of 180 minutes of operation. As seen, the microorganism concentration sharply decreases from the frequency of 20 kHz to the frequency of 100 kHz, at which it reaches the minimum value. The strong impact of the ultrasound frequency on microorganism disintegration continues between 100 kHz and 400 kHz, at which the sonochemical effects are prominent. However, disastrous sonochemical effects are alleviated with the increasing frequency until it reaches 800 kHz. These results indicate that the sonochemical effects are more dominant in microorganism disintegration between the medium frequencies of 40 and 400 kHz. Hua and Thompson, (2000) investigated a wide band of ultrasound frequencies (20 - 1071 kHz) and reported that the highest inactivation was observed at 205 kHz.

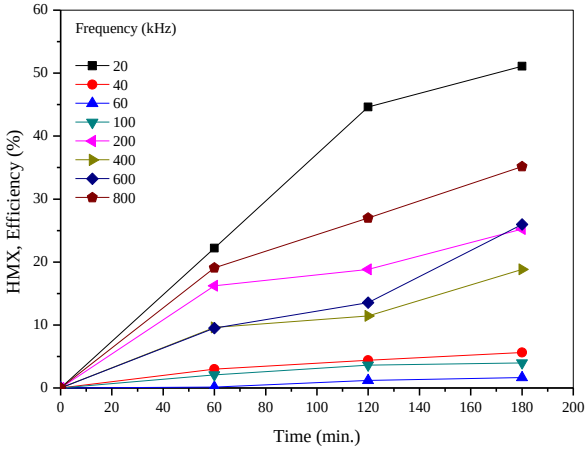
TNT, RDX, and HMX were selected as the pollutants to be treated in this study since they pose serious threats to the environment and human health. These compounds have different physicochemical properties. The nitro group gives these compounds the recalcitrance property to biodegradation (Tiehm, 1999; Ju and Parales, 2010). The reaction mechanism of each pollutant differs according to the physicochemical properties of these compounds (Zhang *et al.,* 2006).



(a)



(b)



(c)

Figure 4. Removal of TNT, RDX, and HMX at different ultrasound frequencies

The preliminary results on the effect of ultrasound on TNT, RDX, and HMX are presented in Figure 4. As shown, the best results all three explosives were obtained at the frequencies of 20 and 800 kHz. The change in the glucose concentration that was used to observe the selective property of ultrasound was so low that it was not taken into consideration.

Within the first hour of the study, the removal efficiency of TNT was very good except at the frequencies of 60, 100, and 400 kHz (Figure 4a). When the frequencies of 20 and

800 kHz were applied, the removal efficiency showed a linear increase. However, the remaining frequencies did not exhibit good removal yields. The efficiency of RDX removal was very low compared to that of TNT and HMX. Similar to TNT, the best results for RDX were achieved at the frequencies of 20 and 800 kHz; however, the increase was not linear. The lowest removal efficiency for RDX was obtained at 60 kHz. The best results for HMX removal were also obtained at 20 and 800 kHz (Figure 4c). Here, it should be noted that the removal efficiencies for HMX, which is the most hydrophobic compound among the three, were higher than the other two compounds. This can be explained by the accumulation of hydrophobic pollutants and their reaction at the hydrophobic boundary layer of bubbles (Zhang *et al.,* 2006). Since the OH• radicals and H_2O_2 concentration at the boundary layer are higher than the bulk solution, pyrolysis and radical reactions can contribute to degradation (Hua, 2000; Zhang *et al.,* 2006). The results obtained at 40, 60, and 100 kHz were very similar for the three compounds. It was also determined that for the removal of TNT, RDX and HMX, the main degradation factor at 800 kHz was oxidation by the OH• radical, however, at 20 kHz, it was pyrolysis

4. Conclusions

Ultrasound irradiation is affected by a combination of complex and interrelated dynamic factors related to the wastewater medium such as temperature, dissolved gases, suspended matter, target molecule structure, and microorganisms present. As a consequence of mechanical and chemical effects of ultrasound, the contaminants in wastewater undergo a series of oxidative degradation processes. Ultrasound irradiation can also accelerate the biological treatment of munitions wastewater when applied at an appropriate frequency and power density. In this study, the frequencies of 20, 40, 60, 100, 200, 400, 600, and 800 kHz (MQ-20/40/60, MQ-100, MQ-200, MQ-400, MQ-600, and MQ-800) were investigated to determine the optimum frequency for the removal of TNT, RDX, and HMX. The best results in terms of both removal efficiency and minimum cell lysis were obtained when the ultrasound frequencies were set at 20 and 800 kHz. Therefore, it is suggested that for an effective ultrasound-assisted anaerobic degradation of munitions wastewater, further research is undertaken to determine the optimum power density and sonication time at the 20 and 800 kHz frequencies.

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