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Screening the Effect of Ultrasonic Wave on Effluent Treatment

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Abstract

The effort was made to screen the effect of ultrasonic waves on the purification of waste water. The ultrasonic treatment was found to be effective for the waste water purification. It was found that low intensity ultrasonic irradiation can improve the microbial activity and thus it can be used to enhance the biological treatment of wastewater and increase the treatment efficiency by improving microbial activity in the biological reactor. The biological effects of low intensity ultrasound and the mechanisms associated with the enhancement of biological activity were discussed. The applications of ultrasonic waves in bioengineering and biochemical studies are comprehensively summarized.

Keywords: Effluent; Ultrasonic waves; Waste water; Media; Bacteria

Introduction

Wastewater is composed largely of the substances responsible for the pathogenic and toxic materials. Processing and disposal of wastewater is one of the most complex environmental problems faced by the engineers as well as scientists in this field. Today, biological treatment systems are widely used in wastewater treatment due to its high handling capacity and less cost. However, with the increasing population and economic development, most existing biological treatment systems cannot efficiently handle the increasing and complicated wastewater. Therefore, numerous wastewater treatment plants have to be reconstructed or extended with high costs and many control techniques to guarantee a constant effluent water quality. When ultrasound is applied to effluent, water undergoes thermal dissociation to H atoms and OH radicals. OH is highly reactive and can oxidise almost all contaminants in water. This primary oxidation is the reason for the degradation of contaminants in water [1-12]. Sono-chemical reactions are normally characterised by the simultaneous occurrence of pyrolysis and radical reactions, especially at high solute concentrations. Volatile solutes undergo direct pyrolysis reactions within the gas phase of the collapsing bubbles or within the hot interfacial (cavity-liquid) region. The bacteriocidal effect of ultrasonic wave has been evaluated on *E. coli* ATCC 10536, *Salmonella typhimurium* ATCC 14028 and *Listeria monocytogenesis.*

Ultrasound is defined as acoustic energy or sound waves with frequencies above 20 kHz. Low intensity ultrasound generally refers to that whose intensity is less than 10 w/cm^2. When ultrasound propagates in sound bearing media, it usually can cause some effects on this media; if the sound bearing media is biological materials, such effects induced by ultrasound are called biological effects of ultrasound. When ultrasound propagates in a biological media, firstly it causes mechanical stresses on microorganism, and then part of mechanical energy converses into thermal energy. If the intensity of ultrasound is high enough, the ultrasound can cause cavitation stresses. Consequently, the biological effects of ultrasound primarily include mechanical effect, thermal effect and cavitation effect.

Effects of Ultrasonic Waves

Vibration effect

The vibration with high frequency induced by ultrasound causes effective agitation in liquid media, which increases the fluid mixing, diffusion and mass transfer of substrate. The boundary layer of stagnant fluid adjacent to a solid surface creates a resistance to the transport of small molecules to the surface. The high-frequency vibration reduces the thickness of this boundary layer, thus increasing the convection transport.

Acoustic streaming effect

When ultrasound propagates in liquid media, it can create the unidirectional and constant sound radiation pressure, which causes the liquid to flow in the direction of the sound propagation. This phenomenon is defined as acoustic streaming, and it increases with ultrasound intensity. Therefore, any amount of ultrasound in a liquid produces additional convection transport from acoustic streaming. Vibration effect and acoustic streaming effect both belong to mechanical effects of ultrasound. These two effects can improve the mass transfer and fluid mixing through the acceleration of convection transport.

Thermal effect

In the process of ultrasound propagation, the media can absorb the energy of ultrasound and converse it into thermal energy. However, in exposure systems such as enzyme solutions and cell suspensions with efficient heat transfer and narrow temperature control, thermal effect would make only a marginal contribution to enhanced bio-reaction induced by ultrasound.

Cavitation effect

Cavitation is a particular phenomenon of ultrasound in liquid media. The molecule of pure liquid has a very high strength of extension. Actually, some minute gas bubbles can enter into the practical liquid for various reasons, which produce the “weak link” of the liquid. The cycles of low and high acoustic pressure causes the gas bubbles to expand and shrink, which in turn creates shear flow around the oscillating bubbles. This process of expansion, shrinkage and collapse of gas bubbles induced by ultrasound is called ultrasonic cavitation, and the minute gas bubbles are called cavitation bubbles. Based on the

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different behaviour of cavitation bubbles, cavitation can be classified as instantaneous cavitation and stable cavitation.

Instantaneous cavitations

It usually occurs when the ultrasonic intensity is beyond 10 w/cm^2. It is produced when the bubble radius is reduced to near zero and collapse during the contraction cycle. The sudden collapse produces high heat (about 5000 K) and high pressure (about 5×10^7 Pa), which would form many free radicals and generate strong ballistic waves in fluid, so that all these can deactivate many enzymes and destroy the cell structure. However, the instantaneous cavitation can decompose the toxic and hard-to-degrade organic compounds in the wastewater, thus reducing the toxicity of wastewater to cells. This is just the theoretical basis for the application of high-intensity ultrasound to wastewater treatment. Nevertheless, as mentioned above, the instantaneous cavitations would be disadvantageous to biological materials, so it can only be used as a pre-treatment technology instead of a direct application in biological treatment of wastewater.

Stable cavitations

It refers to the process during which the cavitations bubbles experience an impulsive motion with small amplitudes when the ultrasound intensity is relatively lower. In this process, the periodic expansion and shrinkage of the cativation bubbles can cause strong flows toward or away from bubbles, which is called acoustic micro streaming.

As to the biological media, cells around the cavitations bubbles would undergo the tangential stresses that induced by micro streaming, causing the displacement of the cell membrane in the tangential direction and increasing the local membrane permeability. Once the increases of the whole cell are superposed, the permeability of the whole cell is enhanced, thus accelerating the mass exchange through the cell membrane. This is of great significance to improvement of mass transport. The transport increases with increasing acoustic intensity as larger and more numerous cavitation bubbles form and the amplitude of oscillation increases. Consequently, the stable cavitation effect is the primary basis of the biological effects, and most effects of low intensity ultrasound are thought to be attributed to, at least in part, the increase of membrane permeability.

Damage effect

The mechanical effect and the stable cavitation effect of ultrasound accelerate the convection transport and improve the membrane permeability, enhancing the uptake of foreign substances and the release of intracellular products in the cells. Therefore, these two effects are widely believed to be the main factors contributing to the ultrasonic enhancement of biochemical reactions [1-17].

Keeping in view the vitality of this task it was taken up to further study to explore the new dimensions using ultra sound waves at 40 and 140 KHz frequency to screen the effect on waste water as a measure for effluent treatment.

Materials and Methods

During the research work, all the chemicals were used of A.R. grade and supplied by E. Merck (India), Himedia (India), S.D. Fine chemicals (India), Qualigens (India) or Sigma (U.S.A). Waste water sample was collected from Paper and Pulp industry, Meerut (U.P.) India.

Ultrasound is simply mechanical waves at a frequency above the threshold of human hearing. It can be generated at a broad range of frequencies (40 and 140 KHz) and acoustic intensities.

Ultra sonic wave conditions

Ultrasonic (US) pre-treatment specification were taken as follows:

Treatment time (min.): 10 (t_1), 20 (t_2) and 30 (t_3); Sample volume: 100 ml; US Frequency: 40 kHz and 140 kHz; US Power: 250 W; US Intensity: power supplied per transducer area (50.95 Watt per cm sq.); US Density: power supplied per sample volume (2500 Watt per lit); US Dose: Energy supplied per sample volume (J /L).

In the present study, the following conditions were useful for the isolation, characterization and treatment of isolated bacteria which were obtained from industry waste water (Table 1).

Media preparation and bacterial isolation

Waste water samples were treated at 40 KHz and 140 KHz frequency for time- 10, 20 and 30 min, respectively and the control (without ultrasonic treatment) had 5 µl of sample spread on LB plates aseptically under laminar air flow and incubated at 32°C for overnight.

Estimation of biochemical oxygen demand (BOD)

Treated sample dilution was prepared in a 300 ml BOD flask.1 ml each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solution was added in 1 liter of sample and pH was adjusted to 7.0. Alkaline potassium iodide, manganous sulphate, starch (indicator) and sulphuric acid (to digest the precipitate) was added in a sample to remove the DO content from sample. Two set of samples were prepared. First set of sample was titrated by sodium thiosulphate solution and second set was kept in BOD incubator at 20°C for 5 days. After the completion of 5 days, second set was also titrated with sodium thiosulphate.

Coli form test of domestic sewage sample

This technique is mainly use for the detection of presence or absence of coliform bacteria in water from treatment plants. 100 ml sample was collected in flask and treated with ultrasonic waves. Sample was transferred into ziplag bag and then in sterile disposable bottle (100 ml capacity). Entire quantity of dehydrated medium (Lauryl Tryptose Broth) was added slowly to water sample by swirling to dissolve the powder completely. After dissolution the sample was incubated at 30-35°C for 24-48 h.

Culture medium for *Escherichia Coli* (*E. coli*)

10 g peptone, 10 g lactose, 2 g K_2HPO_4, 15 g Agar, 0.4 g Eosin and 0.065 g Methylene blue were added in 1 litre of distilled water and maintain pH up to 7.1. Sample was kept for autoclaving and poured in sterilized petri plates under laminar air flow. Plates were kept an incubator for overnight. One drop of diluted sample was spread on the plates and kept it for overnight an incubator.

Ingredients	Amount (g/L)
Yeast extract	5
Sucrose	0.5
NaCl	5
Tryptone	10
pH	7.8

Table 1: LB Medium (Luria-Bertani).

Ultrasound treatment of waste water

Waste water sample of sewage was collected from Paper and Pulp industry, Meerut (U.P). This is followed by ultrasonic treatment at four different duration of time (5,10,20 and 30 min) using ELMA, multi frequency ultrasonic bath (according to manufacture Instruction), The Sonication of wastewater was performed in borosilicate glass vessel (250 ml). During sonication, waste water temperature was increasing gradually with time. pH and electric conductivity of sample were measure by electrode based probe (Water and Soil analysis kit, Electronics India, Model 161E), the rise in temperature of sample on ultrasonic treatment was measure by mercury filled thermometer. In order to characterize this sample pH, electric conductivity, total solid content (gm/l), COD (mg/l), Total Nitrogen, Total Phosphorus, BOD ,Gram staining and *Phosphatase* activity test were measured.

Screening of isolates for *Phosphatase* activity

The basic principle behind the determination of *phosphatase* activity is to supply insoluble phosphorus source in agar based medium for the growth of the bacteria. Use of yeast extract is avoided in the medium. *Phosphatase* activity of all the isolates was tested after growth in Goldstein solid agar medium which is specifically used for screening phosphate solubilizers [18].

Coli form test of sewage sample

This technique is mainly use for the detection of presence or absence of coliform bacteria in waste water by ultrasonic treatment. After 24-48 h. incubation, if colour changes of the medium from redish purple to yellow, indicating the presence of coliform bacteria. But due to the ultrasonic treatment no any population of *E. coli* was present in the sample. It could be because no any colour changes in the medium. If colour becomes light yellow than it shows positive test.

In the Table 2, all the solutions were made separately and autoclaved. Solutions were cooled down to about 50°C. Solution-2 was added to solution-1 and then solution-3 was added to this mixture. The resulting solution was poured into petri plates and was allowed to solidify. After a day plates were inoculated with cultures by streaking. Plates were incubated at 30°C for one week. Plates were observed for the zone of solubilisation of insoluble phosphate (Halozone).

Procedure for *E. coli* test by lauryl media

100 ml sample was collected in flask and treated with ultrasonic waves. Sample was transferred into zip lag bag and then in sterile disposable bottle (100 ml capacity). Entire quantity of dehydrated medium (Lauryl tryptose Broth) was added slowly to water sample by swirling to dissolve the powder completely. After dissolution the sample was incubated at 30-35°C for 24-48 h.

Results and Discussion

Schneider [19] reported that the sludge samples contain millions of microorganisms (bacteria). Ultrasonic shock waves break the microbial cell walls. The intracellular compounds (protein, enzyme and fat) are released in sludge aqueous phase resulting in an increase of chemical oxygen demand (COD). Flym and Flym and Atchley [20] investigated that in a liquid medium, the effect of ultrasound is produced due to phenomenon called cavitation. The result shown that during the negative cycle of the wave, the distance between the molecules of the liquid will vary (oscillate) about a mean position. If the distance between the molecules exceeds the critical molecular distance R eg. For water the value of R is 10^{-8} cm), then the liquid will break down and voids will be created i.e. formation of cavitation bubbles. During the positive cycle of the wave, the bubbles grow in size due to the positive acoustic pressure and then finally collapse, leading to the formation of new nuclei for the next cavitation. In water implosion and fragmentation of the bubble which collapses are the centre of high energy phenomena; temperature, pressure and electrical discharges giving rise to direct destruction of macromolecule of solute and the sonolysis of water to form H° and OH° radicals. OH radical reacts with another °OH (hydroxyl) radical to form hydrogen peroxide. Both H_2O_2 and °OH are strong oxidizing species. On sonolysis of water (aqueous solution) Hydroxyl radical (OH) and Hydrogen atom form as per reactions (i-viii) shown below [19,20].

H_2O - H°+HO° (i)

O_2 - 2O° (ii)

O°+H_2O - 2HO° (iii)

H°+HO° - H_2O (iv)

2HO° - H_2O (v)

H°+O_2 - HOO° (vi)

2HO° - H_2O_2 (vii)

2HOO° - H_2O_2+O_2 (viii)

Biochemical oxygen demand (BOD)

When DO was determined on the very first day, the concentration of treated waste water sample increased and then decreased according to their treatment time. It is observed that of ultrasound frequency at 140 KHz is more effective than at 40 KHz. After 5 days of incubation the concentration of DO decreased, as shown in Table 3, after determining the DO content in wastewater.

where, sample volume was 1% in 1 L, A, B, C refer to sample under the effect of 40 KHz ultrasonic waves after 10, 20 and 30 minutes while A[1], B[1], C[1] refers to sample under ultrasonic effect after 10, 20 and 30 minutes.

Isolation and growth of bacteria: Sludge samples were treated at

Solution	Ingredients	Amount (g/L)
Solution 1	General purpose Agar	20.0 g/l
	Glucose	10.0 g/l
	NaCl	1.0 g/l
	NH_4Cl	5.0 g/l
	$MgSO_4.7H_2O$	1.0 g/l
	pH	7
Solution 2	K_2HPO_4	5.0 g/50 ml
Solution 3	$CaCl_2$	10.0 g/100 ml

Table 2: Medium composition.

Sample	Initial DO (mg/L)	Final DO (mg/L)	BOD
Control	9.50	6.00	350
A (40 KHz)	8.90	5.85	325
B (40 KHz)	8.50	5.80	265
C (40 KHz)	8.40	5.75	255
A[1] (140 KHz)	7.40	6.50	220
B[1](140 KHz)	7.25	6.00	200
C[1] (140 KHz)	7.20	5.60	190

Table 3: Dissolved oxygen of sonicated wastewater after 5 days incubation.

40 KHz and 140 KHz frequency for time 10, 20 and 30 min, respectively including control (without ultrasonic treatment) 5 μl of sample was spread on LB plates aseptically and incubated at 32°C for overnight. Bacterial colonies obtained were counted followed by Gram staining, 40 KHz: A, B, C and 140 KHz: A^1, B^1, C^1. It was observed that the number of bacterial colonies decreased with increase of treatment time and frequency (Figures 1, 2 and Table 4).

Phosphatase activity test: With a view to screen Phosphate-sloubilization activity, simple plate test based, on the formation of halo-zone around the colonies were conducted. Out of six isolates, three isolates showed active phosphate-solubilizing character (Figure 3) [18].

Application of ultrasound in biological waste water treatment: Microorganism is the key factor in the biological wastewater treatment, so we can stimulate the activities of microorganisms to improve the treatment efficiency by the application of low intensity ultrasound. According to above analysis of biological effects and enhancement mechanisms of ultrasound, the proper intensity and irradiation period are two key parameters and should be selected carefully, otherwise the ultrasound would destroy the cells or enzymes. For different bioprocesses, the optimum ultrasonic intensity, irradiation period are different, thus the optimum ultrasonic parameters should be determined carefully by experiments towards different kind of wastewater [1-15].

S. No	No. of colonies at 40 KHz	Temp °C	No. of colonies at 140 KHz	Temp °C	Time (Min)
1.	820	32	745	35	10
2.	735	34	485	37	20
3.	550	37	370	40	30
4.	Control-1525	30		30	

Table 4: Average colonies obtained on LB plates.

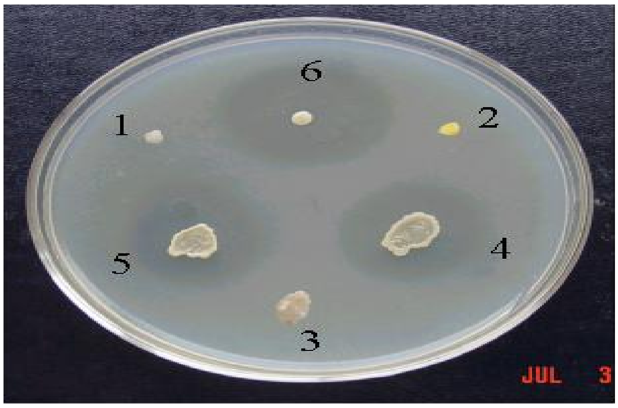

Figure 3: Plates showing halo zones by phosphate solubilizers.

Conclusions

The biological effects and enhancement mechanisms of ultrasound on acceleration of bio-reactions are summarized in this paper, from which we can demonstrate that the application of low intensity ultrasound to biological wastewater treatment is quite feasible in theory. There have been a number of reports on the use of ultrasound to enhance the biological treatment of wastewater. However, most of these studies focused on the optimization of ultrasonic parameters and little attention has been paid to the enhancement mechanisms of ultrasound. Study of the enhancement mechanisms is required not only for understanding the effects of ultrasound but also for more effectively generating the desired effects, which can be great help in practical use.

[A] [B]

Figure 1: Bacterial colonies in waste water sample [A] without ultrasonic treatment [B] with ultra-sonic treatment.

Figure 2: Control and treated sample of *E. coli* bacterial population.

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