Effect of Two Waves of Ultrasonic on Waste Water Treatment

Kumar R[1]*, Yadav N[1], Rawat L[1] and Goyal MK[2]

[1]*Forest Ecology and Environment Division, Forest Research Institute, Dehradun, India*
[2]*National Institute of Pharmaceutical Education and Research, Mohali, India*

Abstract

In the area of water purification, ultra sonication offers the possibility of an efficient removal of pollutants and germs. Ultrasound treatment is one of several technologies that promote hydrolysis – the rate-limiting stage during wastewater treatment. The basic principal of ultrasound is based on the destruction of both bacterial cells and difficult-to-degrade organics. In wastewater, various substances and agents collect in the form of aggregates and flakes, including bacteria, viruses, cellulose and starch. Wastewater is composed largely of the substances responsible for the offensive, pathogenic and toxic materials. In the present research work we found that the ultrasonic treatment is very effective for the waste water purification. The bacterial populations in sludge were decreased according to the frequency (35 KHz and 130 KHz) and time period (5, 10, 20 and 30 min). As the frequency and time period increase the bacterial population was decreased. It was also observed that 130 KHz frequency was more effective than 35 KHz.

This technique would play a major role in sustainable development in a large scale of water purification.

Keywords: Waste water; Ultrasound; Bacteria; Frequency; Cellulose; Sludge

Introduction

Biological treatment processes are widely used in the wastewater treatment industries. However, aerobic and anaerobic treatment processes can result in wastewater that is difficult to treat and handle. Ultrasound treatment is one of several technologies that promote hydrolysis -the rate-limiting stage during wastewater treatment. The basic principal of ultrasound is based on the destruction of both bacterial cells but difficult-to-degrade organics. In wastewater, various substances and agents collect in the form of aggregates and flakes, including bacteria, viruses, cellulose and starch. The energy produced during ultrasound treatment causes these aggregates to be mechanically broken down, altering the constituent structure of the wastewater and allowing the water to be separated more easily, because ultrasound attacks the bacterial cell walls, the bacterial cells release iso-enzymes that biocatalyst hydrolytic reactions [1]. This results in acceleration in the breakdown of organic material into smaller readily biodegradable fractions. The subsequent increase in biodegradable material improves bacterial kinetics resulting in lower wastewater quantities and, in the case of anaerobic digestion, increased biogas production. Therefore, its use is most suited to streams containing large quantities of refractory material and/or cellular matter.

Wastewater are conditioned with polymers to enhance the efficiency of dewatering with presses and centrifuges but the relationship between fluid dynamics and the polymer/ wastewater interaction has been shown to be critical in mixing and conditioning performance[2]. Processing and disposal of wastewater is one of the most complex environmental problems faced by the engineers as well as scientists in this field. Wastewater is composed largely of the substances responsible for the offensive, pathogenic and toxic materials.

Ultrasonic wave in context with bacteria

During the past 20 years the effects of underwater shock waves on living cell have been the subject of money in investigations. Destruction effect of ultrasonic wave on microorganisms and the observed effect in renal infections after extracorporeal shock waves [1]. A possible application could be in a new non-thermal preservation. The bactericidal effect of ultrasonic wave has been evaluated on *E. coli* ATCC 10536,

Salmonella typhimurium ATCC 14028 and *Listeria monocytogenes*. Our result indicates ultrasonic produce pressure variation, cavitation and the radiation resulting from the underwater shock wave reduce the viability of these microorganisms.

Bacterial spores are so resistant to sonic and ultrasonic waves that such treatment used in past to eliminate vegetative cells from suspensions. Another useful application of ultrasonic wave treatment might to break up aggregates. Ultrasonic treatment however induces changes in certain characteristics of spores: swelling occurs, the surface is eroded and growth is stimulated. Some spore may be killed if the treatment is severe enough.

There is no doubt that microbial world constituted of diverse forms of microbes that dwell ubiquitous habitats i.e. water, air, soil, living/ non-living system etc. Attempts were made to probe the stratosphere in the years immediately prior to the space age. Although it was claimed that bacteria and fungi could be found over the altitude range 18-39 km, such results were generally dismissed on the basis of contamination. Several early investigations were undertaken to attempt to determine the relationship between the number of viable bacteria found in the air and various meteorological parameters such as temperature, humidity and wind speed. Since that time more sophisticated techniques for measuring air pollutants and viable airborne microorganisms have been developed. These developments have led to more accurate studies, which show that correlations do exist between viable microorganisms and air pollutants. It is reported that the correlations exist between bacterial density and carbon monoxide, hydrocarbons, nitric oxide, nitrogen dioxide, and sulfur dioxide. Light hart and co-workers investigated the effects of various concentrations of carbon monoxide

and sulfur dioxide on various microorganisms in the laboratory and showed that these agents reduce bacterial density extensively in log-phase cultures and only partially in stationary-phase cultures [3]. Most frequently isolated organisms and their percent of occurrence were *Micrococcus* (41%), *Staphylococcus* (11%), and *Aerococcus* (8%). The bacteria isolated were correlated with various weather and air pollution parameters using the Pearson product-moment correlation coefficient method. Statistically significant correlations were found between the number of viable bacteria isolated and the concentrations of nitric oxide (-0.45), nitrogen dioxide (0.43), and suspended particulate pollutants (0.56). Calculated individually, the total number of *Micrococcus*, *Aerococcus*, and *Staphylococcus*, number of rods, and number of *cocci* isolated showed negative correlations with nitric oxide and positive correlations with nitrogen dioxide and particulates. Statistically significant positive correlations were found between the total number of rods isolated and the concentration of nitrogen dioxide (0.54) and the percent relative humidity (0.43). The other parameters tested, sulfur dioxide, hydrocarbons, and temperature showed no significant correlations [4].

Phosphate solubilising activity

Improvement in soil fertility leads to increase in agricultural and forest production or primary production, which support the heterotrophs. In many cases the total nutrients of each type of soil remain the same even then some nutrients become limiting to the primary producers. These nutrients (elements) get locked in unavailable forms due to biological immobilization and chemical precipitation.

Many soil microorganisms are able to solubilize unavailable forms of calcium bound phosphorus by their metabolic activities by excreting organic acids, which either directly dissolves phosphorus locked in rocks or chelates Ca^{++} to bring phosphorus into solution. The production of microbial metabolites results in decrease in soil pH, which probably plays a major role in solubilization. Besides change in pH, chelation by organic acids, which bind phosphate anions, also bring about phosphate in soil solution [5,6].

Soil inoculation with phosphate solubilising bacteria has been shown to improve solubilisation of fixed soil Phosphate and applied phosphates resulting in higher crop yield. High pH, high salt concentration and high temperature lead to poor growth and survival of phosphate solubilising bacteria. The decrease in pH clearly indicates the production of acids, which is considered to be responsible for Phosphate-solubilisation. It has been found that microorganisms, which decrease the medium pH during growth, are efficient Phosphate-solubilizers [7] suggested that calcium activity is an important factor controlling the rate and extent of dissolution of rock phosphate. In lab condition, maximum solubilization of phosphate occurs after 3 days of incubation of phosphate solubilizing bacteria. Further incubation of up to 5 days does not improve the extent of solubilization.

Mineral phosphate solubilizing (MPS) genes have been cloned from *Erwinia herbicola*. They have been identified as the genes that code for PQQ biosynthesis [8]. The PQQ is required for the functioning of glucose dehydrogenase. This system is involved in energy generating incomplete oxidation pathway that produces gluconic acid, which at the periplasmic space of some bacteria generates protons that functions to dissolve the insoluble phosphate. It is felt that direct oxidation pathway can be exploited to mine the mineral phosphates to release the inorganic phosphate for plant uptake. Two strains of *Rhizobium leguminosarum* bv. *phaseoli* colonizing maize and lettuce root have been shown to have in vitro phosphate solubilizing ability [9].

Materials

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

Methods

Ultrasound is simply mechanical waves at a frequency above the threshold of human hearing. It can be generated at a broad range of frequencies (35 and 130 KHz) and acoustic intensities.

Ultrasonication conditions

Ultrasonic (US) pre-treatment specifications were taken as under [10].

Treatment time (min.): $5(t_1)$, $10(t_2)$, $20(t_3)$ and $30(t_4)$, Sample volume 100 ml.

US Frequency: 35 kHz and 130 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.

Media preparation and bacterial isolation

LB Medium (Luria-Bertani)

Ingredients	Amount g/L
Yeast extract	5.0
Sucrose	0.5
NaCl	5.0
Tryptone	10.0
PH of solution	7.8

Waste water samples were treated at 35 KHz and 130 KHz frequency for time- 5, 10, 20 and 30 min respectively; including control (without ultrasonic treatment). 5μl of sample was 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.

Coliform 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

Figure 1: Control and Treated Sample of *E. coli* Bacterial Population.
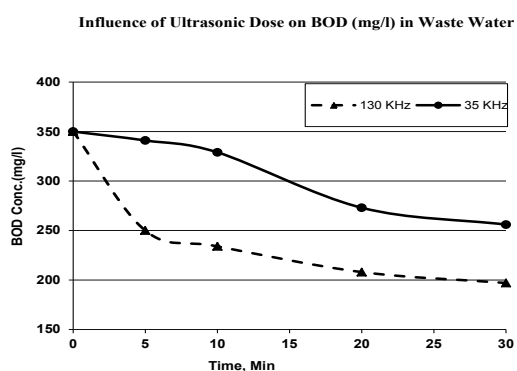

Figure 2: Influence of Ultrasonic Dose on BOD (mg/l) in Waste Water.

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.

Escherichia Coli form (*E. coli*)

Culture medium for *E. coli*: 10 g peptone, 10 g lactose, 2 g KH_2PO_4, 15 g Agar, 0.4 g Eosin and 0.065 g Methylene blue were added in 1litre of distilled water and maintain pH up to 7.1. Sample was kept for autoclaving and poured in sterilized petriplates 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.

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 [9] which is specifically used for screening phosphate solubilizers.

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 reddish purple to yellow, indicating the presence of coliform bacteria which is shown in (Figure 1). 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.

Medium Composition:

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
$MgSO_4 \cdot 7H_2O$	1.0 g/l
pH	7.0

Solution 2	K_2HPO_4	5.0 g/50 ml
Solution 3	$CaCl_2$	10.0 g/100 ml

All the above 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 petriplates 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:

1. 100 ml sample was collected in flask and treated with ultrasonic waves.

2. Sample was transferred into ziplag bag and then in sterile disposable bottle (100 ml capacity).

3. Entire quantity of dehydrated medium (Lauryl tryptose Broth) was added slowly to water sample by swirling to dissolve the powder completely.

4. After dissolution the sample was incubated at 30-35°C for 24-48 h.

Results

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 130 KHz is more effective than at 35 KHz. After 5 days of incubation the concentration of DO decreased, as shown in (Figure 2), after determining the DO content in wastewater.

Bottle No.	Sample vol. in 1 liter	Dilution in %	Initial DO m/l	Incubated	Final DO mg/l	DO drop	BOD
A (35KHz)	10	1	9.25	5 days	5.89	3.36	336
B (35KHz)	10	1	8.85	5 days	5.62	3.23	323
C (35KHz)	10	1	8.45	5 days	5.8	2.6	260
D (35KHz)	10	1	8.3	5 days	5.8	2.5	250
A^1(130KHz)	10	1	9.25	5 days	6.75	2.5	250
B^1 (130KHz)	10	1	7.42	5 days	6.55	2.3	230
C^1 (130KHz)	10	1	7.3	5 days	6.05	2.0	200
D^1 (130KHz)	10	1	7.29	5 days	5.74	1.9	190
Control	10	1	9.5	Untreated	6.0	3.5	350

Table 1: Dissolve Oxygen of sonicated wastewater after 5 days incubation.

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 (Table 2 and Figure 7).

Discussion

All the sample of wastewater was treated with ultrasonic waves of 35 KHz and 130 KHz (Figures 5 and 6). Ultrasound creates large cavitation bubbles which collapsed upon and initiate powerful jet streams exerting strong shear forces in the liquid. The decreasing wastewater disintegration efficiency observed at higher frequency was attributed to smaller cavitation bubbles, which allow the initiation of strong shear forces of comparatively smaller magnitudes. This is the decomposition

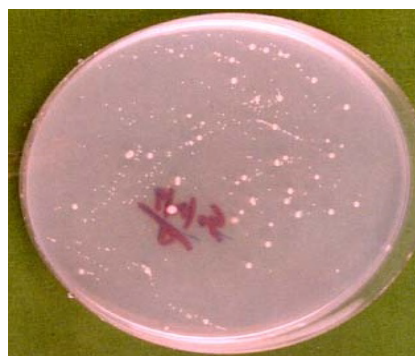**Figure 3a:** Bacterial colonies in waste water sample after ultrasonic treatment at 35 KHz for 5 min.

Figure 3c: Bacterial colonies in waste water sample after ultrasonic treatment at 35 KHz for 20 min.

Figure 3b: Bacterial colonies in waste water sample after ultrasonic treatment at 35 KHz for 10 min.

Figure 3d: Bacterial colonies in waste water sample after ultrasonic treatment at 35 KHz for 30 min.

Isolation and growth of bacteria

Sludge samples were treated at 35 KHz and 130 KHz frequency for time- 5, 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, 35 KHz = A, B, C, D and 130 KHz= A^1, B^1, C^1, D^1. It was observed that the number of bacterial colonies decreased with increase of treatment time and frequency. The number of bacterial colonies are described in (Table 1) and (Figures 3A-3D) A,B,C,D,A^1,B^1,C^1,D^{1}andcontrol (Figures 4A^1-4D^1).

Phosphatase activity test

With a view to screen Phosphate-solubilization activity, simple
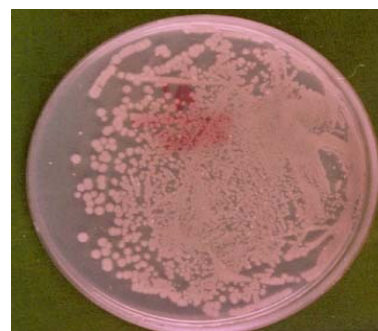

Figure 4a: Bacterial colonies in waste water sample after ultrasonic treatment at 130 KHz for 5 min.
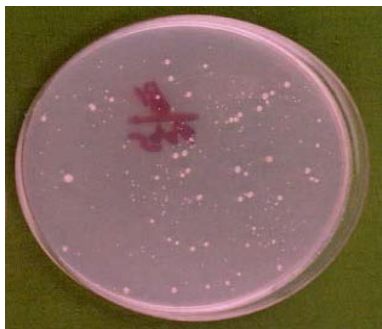
Figure 4b: Bacterial colonies in waste water sample after ultrasonic treatment at 130 KHz for 10 min.

Figure 4c: Bacterial colonies in waste water sample after ultrasonic treatment at 130 KHz for 20 min.

Figure 4d: Bacterial colonies in waste water sample after ultrasonic treatment at 130 KHz for 30 min.

S.No	No. of colonies at 35 KHz	Temp °C	No. of colonies at 130KHz	Temp °C	Time, Min
1.	1086	31	1298	32	5
2.	827	32	756	35	10
3.	742	34	492	37	20
4.	556	37	373	40	30
5.	**Control- 1525**	**30**		**30**	

Table 2: Average colonies obtained on LB plates.

of the flocculent structures within the sludge and or breaking down of the microorganisms embodied in the sludge (Figure 8). Shorter sonication times resulted in wastewater floc deagglomeration without the destruction of bacteria cells. Longer sonication may cause the break-up of cell walls with the probability less for sonication of shorter

duration. It is anticipated that ultrasonic shock waves hits the microbial cell walls. The larger bubbles upon implosion give high mechanical effect, which leads to disintegration of microbial cells. Temperature rise in each sample with the increase in treatment time. Particulate wastewater material was broken down into smaller pieces. We also measured the highest degree of disintegration at 35 KHz. The significant increase of the BOD was attributed to the back up of microbial cells leading to the release of intracellular material [11]. The efficiency of wastewater disintegration decreased with increasing frequency.
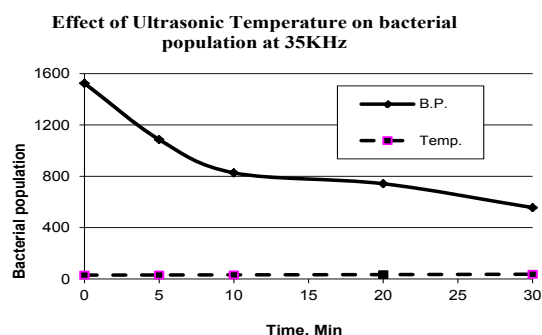

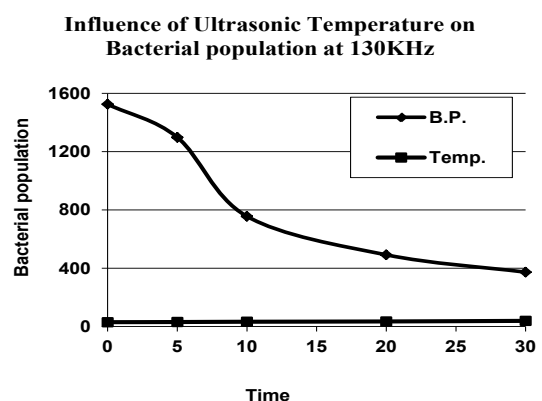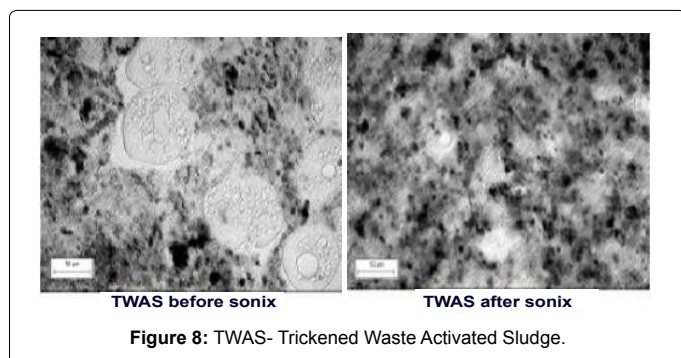**Figure 5:** Effect of Ultrasonic Temperature on bacterial population at 35 KHz.

Figure 6: Influence of Ultrasonic Temperature on Bacterial population at 130 KHz.

Figure 7: Plates showing halo zones by Phosphate-solubilizers. 1. I, 2. II, 3. III, 4. IV, 5. V, 6. VII.

Figure 8: TWAS- Trickened Waste Activated Sludge.

The extent of bacterial cell rupture or cell disintegration were measured on the basis of rate of utilization of dissolve oxygen in wastewater samples. If microbial activities are more the rate of oxygen utilization will be more and vice-versa.

In this experiment, variation of dissolve oxygen in both control and treated samples of wastewater was measured. The dissolve oxygen concentration rates (DOCR) of control sample were more than the treated samples. This shows after treatment bacterial population underwent cell disintegration hence microbial activity decreases so DOCR fell down. The reason behind this is at low treatment time less ultrasonic energy is supplied to the medium (wastewater); due to this deagglomeration of bacterial wastewater flock occurs without the disintegration of bacterial cells. The microorganisms from inside of the wastewater flocs are expressed to the surface and have a better access to the oxygen and hence the oxygen consumption increases.

Conclusion

The ultra-sonication is one of the very useful techniques for the treatment of waste water. Using ultrasonic waves, we can decrease the bacterial population in waste water (paper and Pulp industry, Meerut, Uttar Pradesh, India). Experiment was oriented towards waste degradation through an alternative advanced oxidation technology.

The waste water was treated at two different frequencies (35 KHz, 130 KHz) for different time periods (5, 10, 20 and 30 min). The treated sludge was tested for different parameters (COD, BOD, Total phosphorus and Total nitrogen). Treated sample of waste water was spread on the LB plates, and bacterial isolation followed by morphological identification of Gram staining.

As a result, ultrasonic treatment was found to be treating very effective for the waste water. The bacterial populations in sludge were decreased according to the frequency (35 KHz and 130 KHz) and time period (5, 10, 20 and 30 min). As the frequency and time period increase the bacterial population were decreased. So, it was also observed that 130 KHz frequency was more effective than 35 KHz.

At this premature stage of technique and research beside advantages there are some disadvantages too that must be looked into. After disintegration and anaerobic digestion, waste water has more chemical oxygen demand (COD). So, waste water must be treated.

Acknowledgment

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