Article

Ultrasonic in food microbiology: Application and future trends

Kartikey Chaturvedi[1], **Smriti Chaturvedi**[1,2], **Siddhartha Singha**[3], **Kalyan Das**[1]
[1]Department of Basic and Applied Sciences, National Institute of Food Technology Entrepreneurship and Management, Haryana 131028, India
[2]Institute of Chemical Technology, Maharashtra 400019, India
[3]Indian Institute of Technology, Center for Rural Technology, Assam 781039, India
E-mail: daskalyan27@gmail.com

Received 15 June 2021; Accepted 25 July 2021; Published 1 December 2021

Abstract
Ultrasonic is one of the promising technological innovation to modify structure, inactivate enzymes and/or neutralize microorganisms in food products for enhancement of their quality and safety. Apart from pasteurization, sanitation, disinfection and cleaning procedures, in the area of food microbiology, ultrasound can facilitate recovery of microorganisms and their identification through cell lysis or detachment of microbe from food surface. Hence, study of effect of sound waves on microbial cells in suspended condition and food matrices has opened a new horizon of its application in the area of food microbiology. Use of ultrasound in microbial analysis is already in practice and expanding, but the physics of interaction of acoustic waves with microbial cells in presence of actual food matrices need further attention. Ultrasonic reactor (UR) design needs interdisciplinary approach to further exploit the promise of ultra sound in food microbiology. Substantial demand in recent years for bench-top ultrasound reactors for cleaning of food contact surfaces in common households has increased. This review deals with the state of art of the ultrasound technology, process development, and further scope of the technology specific for its applications in cleaning and sanitization, microbial inactivation and in microbiological analysis in food processing industries.

Keywords acoustic wave; cell lysis; cell separation; microbial analysis; sanitization.

Network Biology
ISSN 2220-8879
URL: http://www.iaees.org/publications/journals/nb/online-version.asp
RSS: http://www.iaees.org/publications/journals/nb/rss.xml
E-mail: networkbiology@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Green technology and minimal processing techniques are the two main concepts which are capturing the interest of both industries and academics researchers. With respect to that, ultrasound (US) fit the criteria the most, being eco-friendly, non-thermal, simple, easy and cost-efficient technique for food processing, cleaning and preservation (Chemat et al., 2011). Either alone or in combination, this non-thermal technique is used as an efficient replacement/complement to the conventional thermal techniques of food preservation and processing. Applications of US for both processing and analysis of food products have been covered in many recent reviews (Bhargava et al., 2021; Awad et al., 2012; Chemat et al., 2011; Wang et al., 2018). However, the interaction of sound wave with microbial cells in different food matrices requires further understanding and therefore relevant research works require to be critically reviewed and chronicled.

Acoustic waves are the mechanical waves having frequency range between 20 Hz and 20 kHz. The waves of frequencies below this range are called infra sound and above this range is called US (Cárcel et al., 2012). Based on frequency range and intensity used, US is either low energy or high energy US. Typical applications of US of different frequency is given in Fig. 1. Low-energy US is also called as low power or low intensity US, and it function at intensities below 1 W/cm sq. and frequencies less than 100 kHz while the high-energy US or high power or high-intensity US have intensities more than 1 W/cm sq. and frequencies from 100 to 500 kHz (Mason et al., 2011; Cárcel et al., 2012; Rosário et al., 2017). In food matrix, the most common range of US used is from 20 kHz to 500 MHz (Chemat et al., 2011).

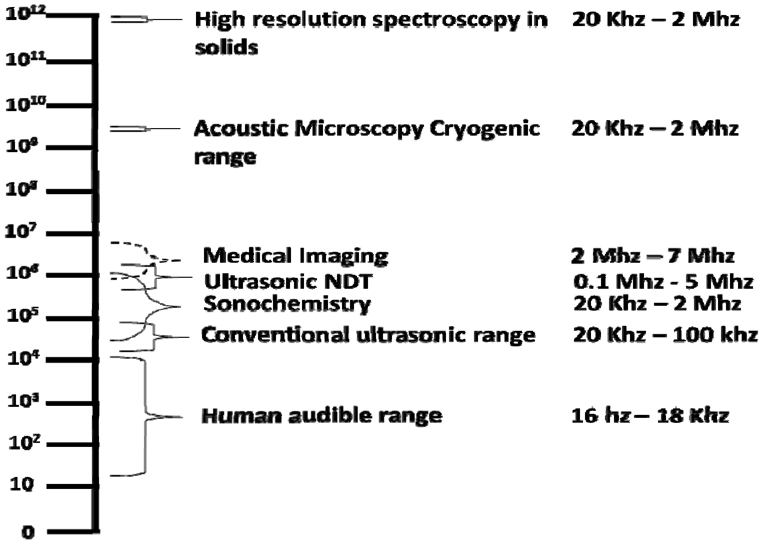


Fig. 1 Ultrasound wave frequency rages and their applications. Picture was designed by the author on Jan 27, 2021.

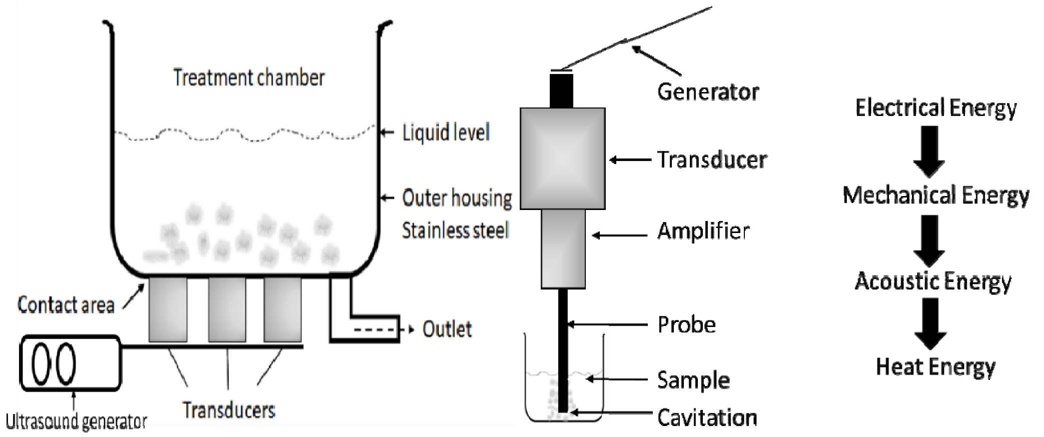
The general principle behind ultrasonication is the phenomenon of cavitation. As soon as the ultrasonic source emits sound waves into a liquid phase, the sinusoidal nature of the waves causes rapid alternating compression and expansion of the liquid particles. Due to this rapid change in pressure, bubbles or voids form in the low-pressure regions of the liquid medium. These bubbles rapidly oscillate in the acoustic field, and beyond a critical pressure, bubbles implode to generate shock waves. The intensity of the process depends on the ultrasonic power and frequency. The cavitation ultimately results in destruction of microbial cells and generation of free radicals. The cavitation is also responsible for bringing desirable change in the foods such as microbial inactivation and structural changes to aid their processing. For example, US promotes release of oils from oil seeds using cavitation (Bermúdez-Aguirre et al., 2011).

This review illustrates the principle behind the concept of ultrasonication, its mechanism, instrumentation models used at the laboratory and industrial level in the area of food microbiology. Comparison of trends and process developments in ultrasound-based sanitization and cleaning processes besides sterilization and pasteurization of various food products have been reviewed. A section has been devoted on the ultrasonic inactivation of various microorganisms depending on food matrices and process conditions. The paper also discusses, the use of US for microbial analysis of food products exploring futuristic developments discussing bio-active extraction with extraction process technologies developed in this decade. It specifies the application of ultrasonication in biofilm removal, cell lysis and cell recovery.

2 Process Engineering of Ultrasonic Reactors

Classical design of ultrasonic reactors consists of three components; electrical power generator, transducer(s), and emitter. The power generator is a source that supplies electrical energy to the transducer, which in turn, transforms electrical energy into mechanical energy. The emitter delivers sound energy into the medium through radiation of the waves.

Most of the laboratory and industrial level 'ultrasonic processors' make use of integrated systems consisting of generator and transducer. Ultrasonic reactors may be bath type and probe type based on the design of sound emitter. The bath type (Fig. 2a) reactors as a result of its early availability, is generally used in food processing for cleaning materials and degassing solutions (Mason, 1998). In case of horn/probe type system (Fig. 2b), the generator, transducer and rod-shaped probe are sequentially attached. Vibration and cavitation are performed by metallic probe (Bermúdez-Aguirre et al., 2011).



(a) Bath type **(b) Horn / probe type**

Fig. 2 Ultrasound wave frequency rages and their applications. Picture was designed by the author on Jan 29, 2021.

US reactors may be batch or continuous type. Batch reactors are suitable to carryout small-scale operations, and mostly used in laboratories. They are easy to operate, flexible and require less labor and energy. They are not used at the industrial level because of the difficulty in scaling up of the efficacy of the system as it depends on processing volume or time (affecting the dissipation of intensity) as previously reported in emulsification of milk proteins (O'Sullivan et al., 2015; Yanjun et al., 2014; Uluko et al., 2015; Cui et al., 2020). Similar challenge was faced by Sucheta et al. (2020) in extracting pectin from black pomace. Long time ultrasonication of the low volume of saccharification media of corn slurry resulted in higher energy densities (Montalbo-Lomboy et al., 2010). In this study, the pre-treated sample slurry was first sonicated using a bench scale reactor, followed by enzymatic hydrolysis in a rotatory shaker incubator at the rotary speed of 150 rpm and incubation temperature of 32°C for 3 h (Montalbo-Lomboy et al., 2010). The batch sonification resulted in higher saccharification yield and 18 times higher energy dissipation rate as compared to that of continuous systems. The energy density requirement of the continuous flow systems to obtain similar product yield as that of batch system was lower. Batch ultrasonic reactor may also be employed to reduce the long pasteurization time in fruit juices so as to achieve 5-log microbial reduction. Baboli et al. (2020) from their study confirmed the same by reporting microbial inactivation of Escherichia coli and Staphylococcus aureus within 0.38 min and 0.55 min, respectively via high intensity fed batch ultrasound reactor. The results were verified by SEM images, thereby concluding that on reducing pasteurization time, the processing costs as well as the negative effects of non-thermal treatment on the nutritional properties of juices minimizes.

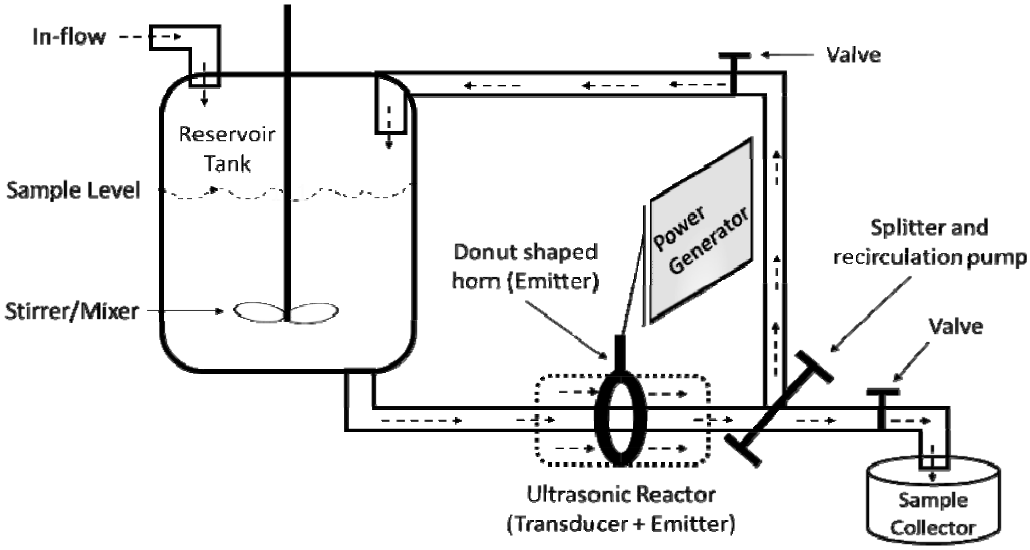


Fig. 3 Schematics of ultrasound equipment in continuous mode. Picture was adapted by the author on Jan 28, 2021 from Boulkhalkhal et al. (2020).

Ethanol industry was the first major food industry to use ultrasonic continuous system (UCS) (Badday et al., 2012). The UCS (Fig. 3) involves use of a “donut” shaped horn, vibrating radially (Montalbo-Lomboy et al., 2010). These horns are deployed in large scale reactors for applications such as waste management operations. In waste management sludge is activated by US increasing the production of methane in anaerobic digestion by increasing availability of nutrient sources for metabolism of anaerobic bacteria (Khanal et al., 2007). In addition to that, continuous US reactor is capable of carrying out heavy duty work for long operation hours unlike the former one. Several researchers have reported increased yield of extraction of essential oils, amino acids, proteins and polyphenols by using continuous ultrasonic than batch conditions (Sucheta et al., 2019; Sucheta et al, 2020; Dujmić et al., 2020). Mohideen et al. (2015) explored the possibility of utilizing continuous flow ultrasonication for pasteurization of blueberry fruit juice. The nutritional and shelf stability studies indicated that increased juice flow rate and amplitude reduced the microbial counts and resulted in high quality juice. The potential of UCS for minimizing the loss of volatile products in liquid foods was studied by Yu et al. (2016). Interesting thin in this study was exploitation of ultrasound assisted Maillard reaction model system for obtaining kinetic parameters and flavor profiles of the product that may allow better temperature control in the reaction at industrial scale and better approaches for developing new food flavors respectively.

3 Ultrasonic Inactivation of Microorganisms: Kind of Microorganism, Food Matrices and Process Conditions

Cavitation is the major cause of microcidal effect of US waves. As discussed, earlier, cavitation (Fig. 4) comprises of mechanical (shear disruption), chemical (free radical formation) and physical (pressure and high temperature) effects as the primary causes responsible for destruction of microorganisms (Bermúdez-Aguirre et al., 2011; Majid et al., 2015). The free radicals formed, attack the cell wall of microbes and causes cell wall disruption. Also, the high localized temperature and high pressure causes the cell to rupture (Table 1).

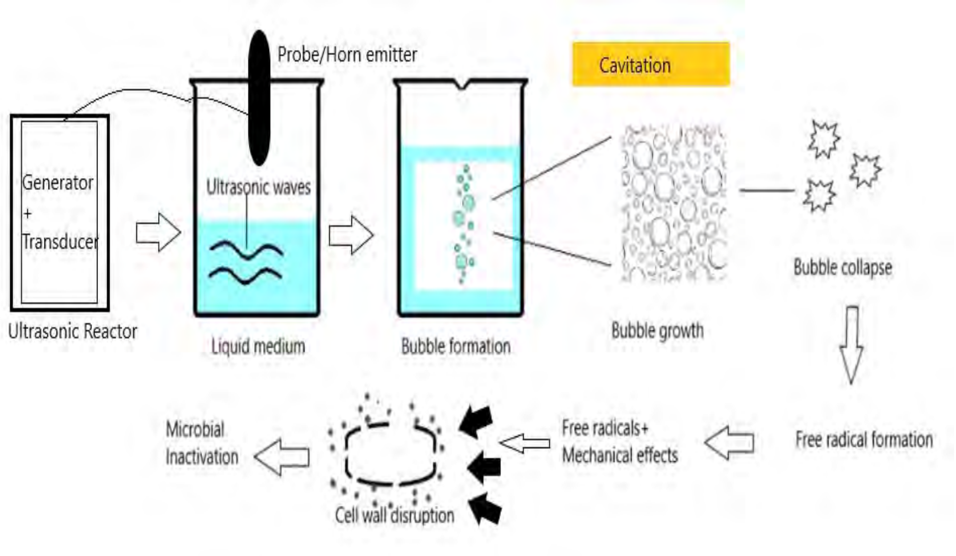


Fig. 4 Microbial inactivation by the phenomenon of cavitation. Picture was adapted by the author on Jan 28, 2021 from São José et al. (2014).

Table 1 Phenomenon of microbial inactivation: causes and effects.

Causes	Effect
Cavitation	• Bubble formation, bubble growth, bubble collapse. • Disruption of cell wall and structures • Cell thinning • Cell wall lysis and release of the cytoplasmic content • Cell breakage, pore formation, and membrane disruption
Free radical generation and mechanical effects	• DNA injury which results in breakages and fragmentation

However, it is often difficult to converge into the actual mechanism of inactivation when US is used with some other factors lethal for microorganisms. The microbial inactivation via US normally follows first-order kinetics when US is considered as the only lethal agent whereas with multiple lethal factors it follows a nonlinear kinetic (Lee et al., 2009). This brings us to the limitation on predicting biological adaption, suggesting necessity on developing more comprehensive knowledge on mechanistic analysis to predict adaptation and growth of microbes during storage. Generally, US frequency range of 200 - 600 kHz is more invasive to microorganisms. Several microorganisms have been studied to establish effectiveness of US (with or without other treatments) for the microbial inactivation (Table 2). Efficacy of such US treatment depends on critical processing factors, such as characteristics of the ultrasonic waves, exposure time, the food matrix, and pathogen itself.

Table 2 Phenomenon of microbial inactivation: causes and effects.

Microorganism	Food	Treatment conditions	Reduction	References
Mesophilic bacteria, mesophilic spores, yeast and molds	Coriander leaves	High power US + supercritical carbon dioxide (scCO2) 40 W; 10 MPa; 40 °C	4 log, 1 log, while yeast and molds <2 log CFU/g, respectively.	Michelino et al. (2018)
Aerobic mesophilic heterotrophic bacteria, total and thermo tolerant coliforms and yeasts and molds	Prebiotic whey beverage	High-intensity US (HIUS) at 53 ± 3°C	2, 2 log and 0.2, 0.4 log, respectively.	Guimarães et al. (2018)
E. coli O157:H7 and *B. cereus*	Brining liquid and beef	US intensity: 20.96 W/cm sq. for 120 min	A significant reduction in number of microbes was observed.	Kang et al. (2017)
L. monocytogenes	Raw salmon fillets	UV + US and UV + US + AEW (acidic electrolyzed water)	0.79 and 0.75 log CFU/g, respectively.	Mikš-Krajnik et al. (2017)
E. coli	Fresh Carrot Juice	US treatment at 58 ^{0}C/ 2 min	No viable cells (>5 log reduction)	Pokhrel et al. (2017)
E. coli O157:H7	Blueberry Juice	Manothermosonication (MTS) (560 W, 5 min, 40°C/350 MPa, 40°C)	5.85-log reduction	Zhu et al. (2017)
S. enterica subsp. Enterica	Strawberry	US treatment (40 kHz, 500 W) at 8°C/ 5 min + Peracetic acid	1.8 and 2.0 log CFU/g reduction	Rosário et al. (2017)
Saccharomyces cerevisiae ascospores	Beer	Thermosonication (TS) at 50°C-1.9 min and TS at 55°C	A significant reduction in microbe count was observed.	Milani and Silva (2017)
L. monocytogenes and *Salmonella enterica serovar Typhimurium*	Fresh-cut bell pepper	Slightly acidic electrolyzed water (SAEW)+US+60°C for 1 min	3.0 CFU/g reductions.	Luo and Oh (2016)
Cronobacter sakazakii	Head lettuce	100 min US and 200 ppm NaOCl.	1.08 log CFU/g reduction	Park et al. (2016)
B. cereus	Potato	3 min of US with 400 W/L of acoustic energy densities	2.3 ± 0.1 log CFU/g reduction	Luo et al. (2016)

		(AED) at 40 °C treatment		
Bacillus cereus spores	Skim milk	Thermosonication (TS) at 24 kHz, 200 W and up to 20 min at 50, 60, 70°C, energy density up to 2.40 kJ/ml	D70°C value was 2.9 min for TS.	Evelyn and Silva (2015)
Total aerobic mesophilic bacteria (TAMB), total coliform bacteria and *Staphylococcus* spp.	Skimmed sheep milk	US at 20 kHz, 78 W for 6 and 8 min and 104 W for 4 and 6 min, energy density 0.62–0.94 kJ/ml	Reduction: >1.81 log CFU/ml for TAMB, complete inactivation for coliforms and >1.6 log CFU/ml for *Staphylococcus* spp.	Balthazar et al. (2019)
Escherichia coli O157:H7 and *Salmonella typhimurium*	Camel milk	US at 20 kHz, 900 W for 15 min; energy density 8.10 kJ/ml	Total elimination of *E. coli* O157:H7 and a 4.4 log reduction in *S. typhimurium*	Dhahir et al. (2020)

3.1 Effect of type of microorganism

The resistance of microorganism to US treatment varies with the type of microorganisms. These variations arise due to differences in cellular structure, envelope and metabolism. 'D' (death rate) value is used to estimate the killing rate of microbes at specific temperature and pressure. When temperature and pressure are kept constant, the resistance of the five types of microorganisms is expressed in D-values in the sequence of: spores > fungi > yeasts > Gram-positive > Gram-negative cells, as a result, the inactivation rate (log CFU/min) is in the order of Gram-negative cells > Gram-positive cells > yeasts > fungi > spores (Feng and Yang, 2011).

Escherichia coli is one of the most studied organisms for US assisted inactivation (Badday et al., 2012; Zhao et al., 2019; Li et al., 2018). Though the US treatment time varied on the food matrix, in general, US of 120 μm 400 W at 24 kHz combined with thermal treatment, ideally at 54°C was found to be most effective (Pokhrel et al., 2017). With a 90% amplitude and thermal treatment at lesser than 56°C, US treatment for 5-10 min resulted in non-detectable levels of *E. coli* cells (Pokhrel et al., 2017).

B. subtilis spores are more resilient than its vegetative cells. Manosonication (MS) treatment of 117 μ amplitude for 12 min at 500 kPa for *Bacillus subtilis* was sufficient for inactivation of both vegetative cells and spores (Raso et al., 1998). This was achieved due to inactivation of spore enzymes. *L. monocytogenes* pathogenic bacterium was most of concerned in ready to eat food product (meat and milk). *Listeria* strains were effectively inactivated via a combination of US with pulsed electric field (PEF) or high-pressure processing (HPP) (Pyatkovskyy et al., 2018). Similarly, ultraviolet light or/and ultrasounds with acidic electrolyzed water, another classic example of combining non-thermal-non-invasive preservation techniques, inactivated *both L. monocytogenes* along and natural microbiota present on raw salmon fillets (Mikš-Krajnik et al., 2017). It is observed that despite extensive works done to minimize food losses by application of US there still limited information available on behavior of microorganisms post the treatment during storage of product. Bacteria especially *E. coli* is well known for its notorious capability of adaption to the chemical treatments. The concerns have been grown exponentially recently due to the proofs emerging for their resilience towards antimicrobial agents (Wang et al., 2020). Therefore, US being a non-chemical method provides a solution however, some extensive works in this direction would be better to support the claims made.

3.2 Effect of processing conditions

It is envisaged that the power of US and exposure time has more trivial role compared to the frequency used for the effective decontamination of the microorganisms in case of minimally processed fruits and vegetables (Seymour et al., 2002). In this context ultrasonic cleaning of fresh produce showed cell removal that were adhered on the surface, more susceptible to the secondary treatment by the sanitizer (Feng and Yang, 2011). Effective destruction of *S. typhimurium* attached on iceberg lettuce were obtained by treatment with US + water and US + chlorinated water than with convectional sanitation techniques (Seymour et al., 2002). Valero et al. (2007) effectively prevented microbial spoilage in orange juice using US with 500 kHz at 240 W for treatment time of more than 10 min. However, treatments of less than 10 min. exposure did not produce evident microbial reduction. In a similar study, commercial sanitizers in combination of US were also studied to evaluate the effectiveness in killing *Salmonella* from minimally processed cherry tomatoes (São José and Vanetti, 2012). The effect of US waves on permeability of membrane is well established, as it contributes in increasing the pore size of membrane, resulting in the increased uptake of soluble gradients, which as consequence lead to inactivation, this is evident from above experiments on *E. coli*. The combined effect of US (at 40 kHz) and ozone (at 1.5 mg/l for 8 min) was successfully applied for effective disinfection of cabbage leaves (Traore et al., 2020). The treatment effectively reduced microbial load from the surface of cabbage while retaining bioactive and antioxidant compounds.

The effects of temperature, pH, organic acids and soluble solids on the inactivation of *E. coli* ATCC 25922 has been investigated using US pasteurization. The study revealed the enhanced sensitivities of *E. coli* to thermal assisted US inactivation (Salleh-Mack and Roberts, 2007). A study based on microbial responses and relation to kinetic modelling is conducted to evaluate inactivation mechanism in *E. coli* cells with manothermosonication, thermosonication, manosonication and sonication. It was concluded that the treatment time required to attain 5-log reduction of *E. coli* was shorter once US was combined with other lethal factors (Lee et al., 2009). Both Temperature and pH show synergistic effect to US treatment, the reason for this is probably they enhance either cell permeability and free radical generation or both. Microbial behaviors are predicted using kinetic models using empirical relationships such as Logistic and Gompertz. These derived parameters are then related with matrix defining parameters such as processing related inactivation (US), temperature and pH for predicting microbial inactivation in food processes (Peleg and Corradini, 2011). Though the stress of environment and processing conditions on pathogens has intrigued attention of most of the researchers, however there is latent lag observed in technologies/models developed to predict the behavior. In this direction Baranyi and Roberts (1994) introduced a mechanistic parameter calling it ‘q0’ explaining the physiological state of cell but recently Peleg and Corradini (2011) argued its empirical nature. This brings back to the same deposition, where in owing to lack of knowledge on recovery and adaptation post to the US treatment.

4 Application of Ultrasound and Microorganism Interaction

4.1 Disinfection of fruits and vegetables using ultrasound devices

Traditionally, chemical sanitizers are used as a common practice for washing fresh fruits and vegetables was considered. The possible reasons for such wider application despite developments in alternative less toxic disinfection processes are: (a) ready availability, (b) easy application, (c) less hassle, and (d) cost effectiveness. However, the inadequate efficiency due to adaptation of pathogens to such chemical agents (Ruiz-Cruz et al., 2007) have necessitated the search for other new strategies. In this regard a very simple solution was evinced where, sonic waves were projected into a cleaning solution, which resulted in cavitation and carryout the microbial inactivation for surface decontamination. This was achieved due to the pressure difference by cavitation a high shear force on the surface of vegetables and fresh fruits, thus micro-steaming removed the entrapped dirt and killed bacteria (Sagong et al., 2013).

Sanitizers are often used along with surfactants for a higher efficacy on microbial reduction. However, chemical nature of such disinfection products and consequence in form of residues is a growing concern among consumers. Therefore, the role of US pre-treatment followed by disinfection with and without surfactants/sanitizers to evaluate decontamination efficiency of fresh produce needs be investigated to understand influence on quality of fresh produce. In this regard Huang et al. (2018) published promising result illustrating that on the application of US alone, significant reduction in the rate of microbial growth was observed as compared to the surfactants. This treatment was performed using a bench top ultrasonic cleaner with ability to maintain constant frequency of 42 kHz and power load of 100 W (Huang et al., 2018).

The effects and efficacy of ultraviolet light (UV 254 nm) and US, two non-thermal process of disinfection on the inactivation of bacteria and changes in color of lettuce and strawberry were investigated (Birmpa et al., 2013). The results revealed that combination of UV and US was efficient in reducing the numbers of mixed microbial population on strawberries and lettuce, indicating that use of these treatments might be employed as a good alternative in comparison to the conventional methods such as chlorine and hydrogen peroxide solutions. The research concluded that UV and US can efficiently replace older methods as presented combination techniques are promising, cost effective and eco-friendly. In another study, on the strawberries, watercress and parsley effectiveness of the use of US combined with chemical sanitizers was evaluated, where a slight color change was observed (São José and Vanetti, 2015). A slight darkening was observed in watercress and parsley, particularly in samples where US was used in association with peracetic acid, while firmness reduction in strawberries. However, the combination of US and 40 mg/l peracetic acid resulted in the highest microbial reduction (São José and Vanetti, 2015). The findings of the study suggested that US treatment might be used an alternative to the vegetable sanitization step.

Another example is of cherry tomatoes, exposing tomatoes to frequency of 45 kHz for 10 min followed by treating with hydrogen peroxide, sodium dichloroisocyanurate, chlorine dioxide and peracetic corrosive significantly improved reduction in microbiota. Among all sanitizers, US coupled with peracetic corrosive indicated the most noteworthy decrease and hence it is reasoned that US is an expected assistant procedure in the disinfection of cherry tomatoes (São José and Vanetti, 2012).

Another added advantage of using US for surface decontamination is that it contributes to antimicrobial effect to the fresh produce. Duarte et al. (2018) confirmed that treating purple cabbage by US + sodium dichloroisocyanurate sanitizer, reduced almost 4 log cycles of *S. typhimurium* adhered to its surface, without altering any physicochemical and sensorial characteristics. A similar study was done by Rosario et al. (2018) wherein the synergistic effect between ultrasound and sodium hypochlorite (NaOCl) effectively reduced the mesophilic aerobic bacteria at 40 kHz, 500 W and 100 mg/l NaOCl for 5 min. Sonolysis was achieved using a 20 kHz ultrasonic unit with an increase in the microbial inactivation on addition of antimicrobial, by means of hydroxyl radicals (Kadkhodaee and Povey, 2008). Recently, an industrial water treatment system has been developed, using high frequency US, patented as Sonoxide, which has shown promising results in microbial inactivation and decontamination purposes (Broekman et al., 2010).

4.2 Food contact surface cleaning and sanitation

The initial applications of US in surface cleaning were majorly associated with removing dirt and microbes from equipment and household appliances (Mason, 2016). Later on, the phenomenon of cavitation along with certain chemical treatments also began to be employed for surface cleaning in food industries as is used for cleaning of fresh vegetables and fruits as well as equipment (Bilek and Turantaş, 2013). The concept of use of power US in combination with in situ generated heat has also been studied recently (Anese et al., 2015) which is unique step in the direction of effectiveness of such combination for water decontamination and recycling in the fresh-cut industry.

Recently a study on chemical and physical effects of acoustic cavitation has been analyzed for the efficient ultrasonic cleaning applications. The study showed that the physical effect of US was useful in ultra-filtration process and for the inactivation of surface pathogens while chemical effects of cavitation were employed for the disintegration of organic pollutants (Yusof et al., 2016). Thus, overall cleaning and sanitization can be achieved efficiently. Another recent research on the novel concept of membrane cleaning via ultrasonically driven bubbles was investigated (Reuter et al., 2017). Here, the idea of damage-free ultrasonic cleaning was introduced, and the results showed that 130 kHz can be used as optimum frequency for carrying out effective cleaning (without compromising the product) at moderate driving powers (~50% of the total power).

The principle of sanitation lies in collapse of cavitation bubbles created by US at solid-liquid interface which, on other hand, resulted in water spray jets and shear forces. The water spray jets and shear forces aided in the removal of dirt or bacteria from the surface. Mason (2016) illustrated this mechanism in a different way, and stated that there are two major factors responsible for the surface decontamination relating to cavitation in an aqueous medium via US in following order: (a) A cavitation bubble ruptures near to a surface, (b) resulting in a powerful jet stream which gusting towards the surface, (c) Detaching bacteria and dirt from the food surfaces. Alternatively, on occasions acoustic streaming occurs when ultrasonic waves displace the cleaning solution. Resulting, in exposure of surface and dirt particles to the liquid stream (increasing sheer force between surface and particles), finally reducing the adhesion force between surface and particle, and thereby providing complete cleaning.

In addition to this, there are certain factors which influence the optimum cleaning by US which includes type of cleaning solution, the presence of acoustic standing wave, bath temperature, power of transducer and the frequency of US. In a study, decontamination efficiency of US for meat was determined which revealed that either alone or in combination with other processing and/or preservation techniques, US hold the capacity for improving the overall quality and ensuring clean and sanitized process equipment at the same time (Turantaş et al., 2015). Moreover, high intensity US can also be used as an effective surface decontamination technique in variety of fish species butas an initial processing step (Pedrós-Garrido et al., 2017). Recently, chemical and physical effects of acoustic cavitation for the prediction of efficiency of disintegration of organic pollutants in water were investigated (BermÚdez-Aguirre et al., 2009).

A typical design of an ultrasonic cleaner consists of a chamber comprising cleaning solution, attached with a transducer which generates the US waves and carryout the phenomenon of cavitation to remove dirt particles (Azar, 2009). In a research continuous-flow ultrasonic washing system was studied to evaluate the effect of surface decontamination on fresh produce (Zhou et al., 2012). The result reveled that US in combination with chlorine, in the continuous-flow system showed enhanced log reductions and decreased total microbial count. Zips et al. (1990) studied ultrasonication for foul smell removal from food processing equipment in a diary industry. US gave reproducible results when applied for 10s for biofilm removal and was four times greater effective as compared to swabbing method. Furthermore, equipment used for food processing could be more effectively cleaned deep inside at holes, corners, rough surfaces or cavities.

4.3 Detachment of bacteria from food matrices

US has also found its application in the biofilm removal (Fig. 5) or bacterial detachment procedures. It removes different types of microorganisms like fungi, bacteria and viruses more efficiently in less time as compared to traditional heating methods (Chemat et al., 2011). Variations in US frequency and intensity and treatment time has different influence on bacteria. Zips et al. (1990) studied the effect of ultrasonic waves applied via ultrasonic bath, for the detachment of microbes adhering to different membrane surfaces. The uniqueness of their analysis was that they observed complete detachment compared to other studies where only log reduction was used as means of comparison of the efficacy of the treatment. Thus, it is conclusive that shear forces across the acoustic layer are the primary factor causing bacterial detachment.

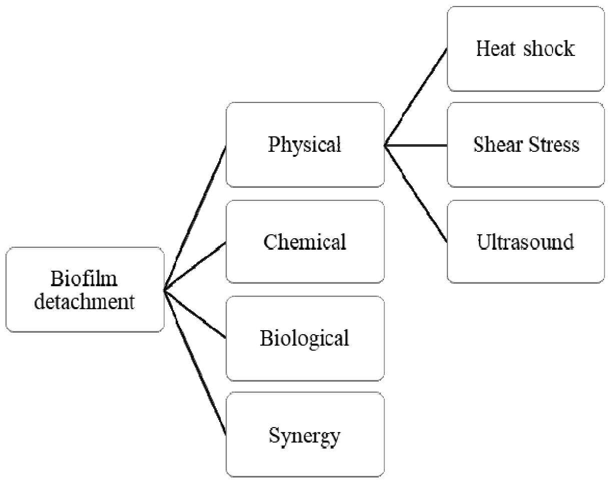


Fig. 5 Different methods for biofilm removal. Picture was designed by the author on Jan 30, 2021.

US along with vortex is used for the in vitro removal of *Salmonella spp.* Biofilms showed that both techniques are equally capable of causing bacterial detachment (Webber et al., 2015). Combination treatment of US and vancomycin reduced the overall thickness of *S. epidermidis* biofilms (He et al., 2011). The use of combination of US and organic acids on lettuce leaves to reduce *S. Typhimurium*, *E. coli* O157:H7 and *L. monocytogenes* was investigated to be more effective than single treatments (Sagong et al., 2011). The synergistic effect of US and peroxyacetic acid can also be used to minimize *Cronobacter sakazakii* biofilms present on fresh-produce like cucumbers and enhancing their shelf-life during transportation without hampering the physical and textural properties of food (Bang et al., 2017).

Chemical sanitizers were coupled with US to see the decontamination effectiveness in strawberries, watercress and parsley. Peracetic acid @ 40 mg/l and US resulted in the maximum decontamination of natural flora as compared to other chemicals (São José and Vanetti, 2015). US has the ability to control microbial contamination and promote decontamination procedures in fruits and vegetables without actually altering any of their quality attributes.

4.4 Cell lysis / bacterial cell destruction for PCR

Cell lysis or cell disintegration is an essential procedure for investigating the detailed information about the cell contents as used in proteomic research and DNA/RNA/protein extraction. The probe-type ultrasonicators or homogenizers are the most commonly used devices. The homogenizer induces vibration in a titanium probe that is immersed in the cell solution (Bermúdez-Aguirre et al., 2011). Cavitation produces high localized temperature, and it disrupt the cell walls by pressure change. This method is extensively used in cell lysis of plant and fungal cells. Cell lysis is also required for molecular detection of microorganism as it causes nucleic acid release. Since nucleic acid (DNA/RNA) extraction can directly show the amount of lysis occurred, it is a techno-friendly tool for easy detection. Sonication based cell lysis is carried out using a bench top lab device and later the lysis efficiency was calculated using real-time PCR. The C_T (calculated threshold) values for PCR amplification of lysed samples showed negligible PCR inhibition or other secondary effects (Vandeventer et al., 2011).

Another potential of US is cell softening. This was examined using apple tissue subjected for 7.5, 15- and 30-min US treatment while the cell wall stiffness was used as performance index, detected using an atomic force microscope (Pieczywek et al., 2017). In the experimental setup the tissue regions of interest were sliced and stained the subjected to a probe-type sonicator with frequency of 40 Hz and peak power capacity of 400 w with 20% of amplitude, pacing samples on slides/metal plates. The image analysis from atomic force microscope revealed a linear decrease in cell wall stiffness. This may have resulted primarily because of increased cell wall permeability, facilitating the transport of solute and water. Thus, it was concluded that US may be used as a cell softening treatment in fruits and vegetables.

Strawberries were US (60 W, 33 kHz) treated to evaluate the effect of treatment on physico-chemical, microbial and nutraceutical quality for the storage period of 15 days at 4 ^{0}C (Gani et al., 2016). The result of the study showed that total soluble solid (TSS) content increased as cavitation via US treatment lead to disruption of cellular structure and formation of microscopic channels that increased dehydration (Fernandes et al., 2009). The increased total phenolic content (TPC) was attributed to greater disruption of cell wall material. The use of low frequency non-focused ultrasound (LFNFU) and high-frequency focused US (HFFU) in microalgal cell disruption was also investigated in a study (Wang et al., 2014). The result depicted that combining high and low frequency treatments is even more useful than single frequency treatment when processing time was kept constant (Wang et al., 2014). Thus, changing and combining frequency can be used in cell disruption.

4.5 Cell separation

The main aim of ultrasonic separation is to achieve efficient enhancement rate of separation processes without altering the chemical or physical integrity of the food product. In recent time, the use of high-power US has found application in cell separation from biomass. Cell separation by US caused clots in target cells that were separated from the system by physical manipulation, particularly by varying frequency (Coakley et al., 2000). The requirement of superior cell concentrations and low sample volumes as well as the lack of appropriate recovery efficiency data or less bacterial cell viability knowledge limited the application of ultrasonication in concentration and separation of cells. This process is called acoustophoresis where the separation of cell or particles is carried out when the sample is exposed to the fluid under the influence of ultrasonic waves. This can be achieved by standing US wave over the cross-section of a microfluidic channel (Gossett et al., 2010).

Juliano et al. (2017) emphasized “the mechanism of separation technology centers around differential positioning of distinctive particles or droplets across US wave field spread within the reactor, whilst actual separation is catered by predisposition of rapidly agglomerating or coating particles”. This technology is being used by oil industry like olive oil, palm oil and coconut oil for oil recovery. High power US assisted cell separation is also used for separating milk fat and for fat globule fractionation (Terefe et al., 2016). A study has also utilized the application of US for transportation of micro-sized particles or cells from stream of one fluid to another, evaluating the separation of polystyrene microbeads of different sizes (3 μm and 10 μm) and waterborne parasites (Cryptosporidium parvum and Giardia lamblia) (Liu et al., 2012). In a study on red radish, use of US assisted freezing resulted in reduced cell separation and disruption of radish tissues (Xu et al., 2015). Several acoustic separation devices have been used in industry. The combination of dielectrophoresis (DEP) and ultrasonic waves causes a series of reactions in particles, i.e., trapping, sorting, concentration and separation with selectivity level up to 90% (Wiklund et al., 2006). Free flow acoustophoresis (FFA) may be used for continuous separation of heterogeneous solution by applying acoustic forces where separation is done on the basis of particles size and density.

Irrespective of the numerous contributions of ultrasonication in food industry, this technique for cell separation and manipulation via acoustic waves finds a much important role in the field of medical science. Acoustic based separation has been used in circulating tumor cells for cancer biology studies (Li et al., 2015), blood cells separation (Kapishnikov et al., 2006), evaluating the behavior of particles mimicking human cell (based on size) under the influence of ultrasonic acoustic field, using micro particle image velocimetry (PIV) measurement technique (Li and Kenny, 2004).

5 Recent Developments and Future Perspective of US Technology

US assisted washers, cleaners, sterilizers and handheld sanitizers have been developed. US treatment is introduced in the cleaning procedures of household items. Ultrasonic ozone vegetable fruit sterilizer-cleaner-washer (Samson multipurpose ultrasonic washer) is a commercially exploited design where the contaminating chemicals from the surface are removed by the action of US and reactive oxygen. The work of ozone is to eliminate odors while chlorine removes adhering microbes. Another equipment is Erngreener handheld ultrasonic fruit and vegetable cleaner which performs surface decontamination in few minutes. Simple, user-friendly, cheap, and being light weight are the added advantage of this US reactor designs.

Many commercial brands of ultrasonic cleaners for domestic user are available like Ultra-waves across the UK, used for cleaning extrusion dies, grillers and choppers. TOVATECH® and OMEGASONICS® originated in U.S.A etc. are popular commercial sonication brands being used to homogenize liquids which extended its application for food contact surfaces or coatings. SONO TEK CORP patented in 2008 an ultrasonic atomic nozzle for spraying coating solutions on food surfaces or food packaging materials. The coating solution might include an anti-microbial solution, an anti-enzymatic browning solution, an edible oil, a liquid flavoring, a liquid spice, a nutraceutical, a protein solution, a peptide solution, a glaze, an anti-stick baking pan release solution, a sterilant, hydrogen peroxide, a food-grade acid, a food-grade propionic acid, alcohol, malic acid, adipic acid, lactic acid and ethanol. The advantage of ultrasonic cleaning over the conventional cleaning processes is that it may reach crevices that are not easily accessible. Also, variety of things can be cleaned via ultrasonic cleaning such as large food packaging crates, process equipment, delicate sensitive medical tools etc.

These recent innovations in US technology undoubtedly brought down large sized industrial technology to small bench top models for wider user groups and application. However, there is still more to explore the true potential of US, which requires modification in instrument to accommodate variable sweeping frequency. The current models are constrained in this regard due to the restriction on the US emitter (horn type) where in mono-emitter operating on single frequency limits applicability of the US. Another challenge is the ohmic heat generated during US process, which increases the temperature of the product as a result may alter the product characteristics effects (Vandeventer et al., 2011). Conventionally it is regulated by introducing sample into temperature regulated water bath or immersing it in a bucket of crushed ice, which at certain times not so convenient and is limited to batch processes. In perspective of continuous process, the energy consumption increases requiring sample cooling and running of operation at lower temperatures. A possible solution is perhaps utilization of Peltier cooling systems for small scale bench top models. Hopefully, future US reactors will be able to overcome these limitations and provide substantial user-friendly bench top models for both commercial and domestic usage.

Apart from the perspective of innovation in US technology and its application one an interesting area to further explore would be impact of US processing on microbial behaviors. Inherently all biological processes are complex and their generalized representation using mathematical relationship is already a challenge. Therefore, predicting microbial behaviors due to additional stress created by US treatment would be an interesting area to explore. This will enable us for developing a better understanding on underlying adaptation mechanism of pathogens due to physiological stresses such as of US.

6 Discussion and Concluding Remarks

Undoubtedly ultrasonication is among the most promising novel method of processing and analysis serving the food. The most successful commercial application of the technology in food sector is probably ultrasonic cleaning and sanitizing devices for both food contact surfaces as well as for fresh vegetables and fruits. Large scale pasteurization is definitely one of the potential areas to replace traditional thermal technologies for better quality liquid food products. In the engineering side now, ultrasonic equipment manufacturers are manufacturing ultrasonic horns of 500 W to 16 kW capable of handling flow rates up to 50 cubic m/h. Current review observes most liquid food processing studies are done in lab scale using batch reactors so pilot level studies are required to scale-up the technology. For solid food products still, much emphasis should be given in ultrasonic bath reactor designs to improve process efficiency and economics to match industrial requirements. Similarly, the recent pandemics and food borne illnesses has resulted in increased demand of such non-chemical microbial inactivation technology. The futuristic development in US requires commercial production of 5-10 litre small scale US reactors for household use. Such bench top models would assist in prevention of food borne related outbreaks.

Further work is also required in the segment of food microbiology, especially when it comes to the cell separation and cellular lysis. Often there is ambiguity in available literature regarding quantification of the extent of cell separation from food matrix. Perhaps more interdisciplinary approach is warranted; along with food scientists and microbiologists engineering principles are required to optimize the analytical process.

Acknowledgments

The support from Department of Basic and Applied Sciences, NIFTEM and NIFTEM Knowledge Centre (NKC) is greatly acknowledged. Kartikey Chaturvedi gratefully acknowledges NIFTEM for PhD fellowship Grant No. PhD/15-16/7/136 and Ministry of Food Processing Industries (MoFPI), Government of India. The author also acknowledges valuable insights and suggestions provided by Dr. Prasanna G.V., Professor, Department of Agriculture and Environmental Sciences (AES), NIFTEM, Kundli, India.

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