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# A novel hybrid cavitation process for enhancing and altering rate of disinfection by use of natural oils derived from plants

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## Abstract

The present study is an attempt to improvise the hydrodynamic cavitation methodology for effective disinfection of water and also to suggest prototype development for practical application. The enhancement in the disinfection efficiency was evaluated specifically for the effect of pressure, temperature, pH, microbial inoculum size and also on effect of different additives for the two model microbial strains, gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*). The efficacy of the hydrodynamic cavitation is evaluated for the two types of flows/ cavitation devices – linear flow in the case of orifice and vortex flow for vortex diode. The vortex diode requires significantly lower pressures, 50% lower as compared to orifice for the similar extent of disinfection. While the bacterial disinfection at high temperature is known, the usefulness of hydrodynamic cavitation is especially evident at ambient conditions and the process is effective even at very high concentrations of bacteria, not reported so far. The reactor geometry also has significant effect on the disinfection. The present study, for the first time, reports possible use of different natural oils such as castor oil, cinnamon oil, eucalyptus oil and clove oil in conjunction with hydrodynamic cavitation. The nature of oil modifies the cavitation behavior and an order of magnitude enhancement in the cavitation rate was observed for the two oils, eucalyptus and clove oil for a very small concentration of 0.1%. The increased rates of disinfection, of the order of 2-4 folds, using oil can drastically reduce the time of operation and consequently reduce cost of disinfection. A possible mechanism is proposed for the effect of oil and hydrodynamic cavitation in cell destruction through the rupture of cell wall, oxidative damage and possible DNA denaturation. A cavitation model using per pass disinfection was used to correlate the data. The increased efficiency using oils and possible benefits of the developed process, where natural oils can be perceived as biocatalysts, can have significant advantages in practical applications.

*Key words: Wastewater treatment, Disinfection, Cavitation, Oil, Antimicrobial*

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## 1. Introduction

The quality of water has been a major concern worldwide and Asia is one of the worst affected region facing the problem of water contamination with pathogenic bacteria [1]. In addition, scarcity of drinking water also has become a major issue worldwide due to increased human population and industrialization. The scarcity of water can be alleviated to a certain extent by recycling and reusing the water. However, environmental pollution due to industrial effluents/ sewage water /biological pollutants impacts the quality of water. The biological pollutants are known to cause various water-borne diseases such as amoebiasis, shigellosis, cholera, typhoid fever, Hepatitis A or E and so on [2], consequently reflected in increasingly more number of deaths because of consumption of unsafe drinking water. Thus, there is an urgent need to formulate newer methodologies that are easy to implement and can generate safe drinking water. According to various norms, the desired total coliforms organism in drinking water should be zero. To comply with this stringent regulation, it is necessary to develop an efficient, green and viable technology for water treatment [3].

Though a number of conventional treatments such as chemical and physical water disinfection methods have been used for microbial decontamination, most of these have limitations/ drawbacks resulting into inadequate efficacy translating into limited applicability [4,5]. The conventional physical methods include heating, radiation, microwave, filtration, UV irradiation and plasma. Many of these, though effective, have scale-up problems, high cost and long treatment time. UV irradiation typically has insufficient light scattering ability and is ineffective towards bacterial photoreactivation repair mechanism [6]. Membrane technology for water disinfection also have operational difficulties along with fouling problem, many times requiring frequent replacement of membrane thereby increasing the cost of treatment. Chemical treatment methodologies such as chlorination and ozonation, though have been widely used with ease of scale-up, have been considered environmentally not friendly in recent years due to unpleasant smell and by-products of the disinfection process can be mutagenic and carcinogenic in nature [7]. Some of the disadvantages of chlorination methods can be eliminated using adsorption technologies employing newer adsorbents/ nanocomposites that are capable of eliminating bacteria [8, 9, 10]. Chemical methods are also unable to decontaminate bacteria from water because of mass transfer limitations and are unable to remove biocides resistant bacteria, bacteria residing in biofilm or any sediment [7].

Hydrodynamic cavitation is believed to be one of the most suitable physico-chemical process for disinfection and is gaining attention in recent years. It has several advantages such as ease of operation, easy scale-up, cost effectiveness, no production of harmful byproducts, representing greener approach and one that can work without use of harmful chemicals [11].

The principle of cavitation involves formation, growth and collapse of cavities of bubbles which is facilitated by specific cavitating device. The collapse of cavities is such that it generates extreme conditions of pressures (~1000 atm or more) and temperatures (~5000 K or more) at the point of implosion and as a consequence hemolytic cleavage of water resulting into generation of hydroxyl radicals takes place and these oxidizing hydroxyl radicals participate into chemical oxidation of organic species [12,13]. There can be *insitu* generation of hydrogen peroxide, another oxidizing agent. The overall process is complex and intimate details of generation of different species ( $\text{HO}\cdot$ ,  $\text{H}\cdot$ ,  $\text{HOO}\cdot$ ,  $\text{HO}_2\cdot$ , and  $\text{H}_2\text{O}_2$ ) and subsequent reaction pathways are less understood. Though, in principle, cavitation is classified into four types on the basis of mode of generation of cavities: acoustic, hydrodynamic, optic and particle; from water treatment point of view, only acoustic and hydrodynamic cavitations are considered to be most promising. In the case of acoustic cavitation, cavities get generated by inducing ultrasound waves in the liquid medium ( $> 16$  kHz), while in hydrodynamic cavitation, it is achieved by realising low pressure regions (using small constrictions, rotational flows or their combinations) in the flowing fluid. Although significant work has been reported in the area of sonochemical reactors and its application in water disinfection for real life is practically invisible due to the reasons of high cost of treatment (capital investment as well as power consumption) and difficulties related to scale – up. The cavitating device can be simple linear flow based venturi or orifice or rotational flow based device such as vortex diode [7,11,13,14,15,16]. Most of the studies were carried out using single and multiple hole orifice plate or venturi for water disinfection [17,18]. However, the study of effect of various parameters such as temperature, pH and inoculum size on hydrodynamic cavitation has been reported largely for organic pollutant degradation using conventional devices. Sun et al. [19] reported rotational hydrodynamic cavitation reactor and disinfection efficiency of reactor towards *E.coli* removal. Cerecedo et al.[20] explored various geometries of the cavitation channels between rotor and stator for disinfection of large numbers of *E.coli* and *E.faecalis* bacteria. Madge and Jenson, [21]used 20-kHz ultrasound unit for disinfection of domestic wastewater and found that the disinfection of fecal bacterial efficiency increased with ultrasound power. A number of hybrid techniques have also been reported for disinfection mainly for hydrodynamic cavitation, acoustic cavitation, hydrogen peroxide, ozone, UV etc. and for different reactor geometries [14,15,22, Chand et al., 2007]. However, there are several limitations of conventional devices and efficiencies were not very high. Further rotor based devices and methodologies are expensive and impose higher operating/ maintenance costs [5,23]. The philosophy of enhancing performance of conventional hydrodynamic cavitation for disinfection relies on either intensification using ozone, hydrogen peroxide etc or by process integration with method such as UV both approaches depict only incremental benefits at rather increased cost. Natural oils can have antibacterial, antifungal and antiviral and antioxidant properties [24] and find use in various applications such as food preservations, aromatherapy and fragrance industries. The antibacterial properties are largely due to the [high contents of oxygenates \(Phenolics/alcohols\)](#).

The antibacterial properties of a large number of essential natural oils such as Eucalyptus, clove oil have been well reported [2,25,26, 27]. However, there are no reports showing systematic study on disinfection of pathogenic bacterial from water through addition of natural oil, for real life application or in cavitation. The addition of natural oil in cavitation is expected to enhance and/or alter disinfection process and hence can be suitably used. Further, application of natural additives such as oils can also reduce the cost of operation. Thus, it is instructive to evaluate effect of additives such as oils having disinfection properties in conjunction with cavitation for increased rates of disinfection and for improved efficiency.

In the present study, we explore a newer form of process, for the first time, to provide proof of concept for hydrodynamic cavitation using different natural oils and for different reactor geometries for two model microbial strains- gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*). A newer form of cavitating device, vortex diode and gram-positive *S. aureus* bacteria were investigated in detail and the generality of results was also confirmed using conventional type of cavitation device-orifice and more commonly reported gram-negative bacteria, *E. coli*. Different oils such as clove oil, eucalyptus, cinnamon and castor oil have been studied for their impact on cavitation. A plausible disinfection mechanism was evaluated to confirm the role of cavitation and oil in cell destruction. A cavitation based model using per pass disinfection was successfully applied. The developed newer method is expected to provide practical, low cost and improved operation for complete destruction of bacterial cellular structure/ death of cell. The results of this work would also lead to newer designs of cavitation reactor and easy scale-up.

## **2. Materials and Methods**

### **2.1 Materials**

*Staphylococcus aureus* (ATCC-6538) and *E. coli* (ATCC-8739) were obtained from NCIM-National Collection of Industrial Microorganism at CSIR, National Chemical Laboratory, Pune, India. The different natural oils such as clove oil (Scientific Name: *Syzygium aromaticum* MW 205.642, Boiling point 250 °C, Solubility 2460 mg/L at 25°C, density 1.0652 g/cc at 20 °C), Nilgiri oil (Scientific Name: *Eucalyptus globulus*, MW 154.23, Boiling point 176.4, solubility 3500 mg/L at 21°C, density 0.9267 g/cc), Castor oil (Scientific name: *Ricinus communis*, MW 933.45, Boiling point 313 °C, Solubility less < 1 mg/mL at 68° F), Dalchini oil (Scientific Name: *Cinnamon verum*, MW 282.383, solubility 1 volume in 3 volumes of 70% ethanol at 20 °C, density, 1.052-1.070 g/cc) were procured locally and used as it is without any prior treatment.

### **2.2 Cavitation reactors**

A vortex diode (66 mm chamber diameter) of 1 m<sup>3</sup>/h nominal capacity of CSIR-NCL design (US9422952B2, 2016) was used as a cavitating device for vortex flow based cavitation. Another cavitating device of conventional type, orifice was also locally made using 3 mm diameter single

hole for linear flow based cavitating device. Details of experimental set-up and operation are provided in the experimental section and Fig.1.

### 2.3 Bacterial cultures growth

Bacterial cultures were grown on 50 mL Nutrient Broth (Himedia Nutrient HiVeg broth); incubated at 37 °C, 200 rpm in an incubator-shaker for overnight. The incubation was given to the mid-point of bacterial log phase, which was determined by UV-VIS spectrophotometer at 600 nm, to ensure bacterial population is in robust stage of growth and not in saturation or death phase. The known concentration of bacterial culture was added to the 20 L of water to obtain final concentration of  $\sim 10^4$  CFU/mL.

The number of viable bacteria present in the system was estimated by plate count method. A sample of 10 mL was withdrawn from the cavitation tank at regular intervals of 15 to 60 min for spreading to sterile petri dish containing N. agar medium. The plates were incubated at 37°C for 24 h, and the colonies were counted as colony forming unit per milliliter (CFU/ml).

$$CFU/ml = \frac{\text{Number of colonies on N. agar plate}}{\text{volume plated (mL)}} \times \text{dilution factor}$$

### 2.4 FE-SEM and TEM analysis

The Field Emission Scanning Electron Microscopy (FESEM, FEI-Nova NanoSEM-450) was carried out to observe the morphological changes of bacterial cell after disinfection and to prove efficacy of cavitation treatment. Samples were withdrawn at different time intervals (0 min: before cavitation, with oil treatment and after 60 min of treatment) and were fixed with 4% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 1 h, subsequently washed with phosphate buffer (0.1 M) for 10 min. Further, the fixed samples were dehydrated by using graded series of ethanol solutions (30, 50, 70, 90 and 100% ethanol) for 30 min each before the analysis.

TEM (Transmission Electron Microscopy; Tecnai G2 20 STwin; LaB6 filament as the electron source) analysis was carried out for detailed examination of disinfection process and to study effects of combined cavitation with oil treatment with respect to cell destruction and cell death.

### 2.5 Disinfection using hydrodynamic cavitation

A schematic of the hydrodynamic cavitation using different cavitating devices is shown in the Fig. 1 along with the photograph of experimental set-up housing the different cavitating devices and the present work focuses on two devices namely orifice and vortex diode. The essential components of the experimental set-up include a high pressure multistage centrifugal pump, a 50 L volume water storage tank, cavitating devices, temperature control (JULABO Chilling system, Model FL 1701, 20 L); pressure and flow controls/indicators etc. The details of experimental set-up are described in our earlier publications [12,13,16] therefore have not been repeated here. Typically, 20 L volume of contaminated water was used for each experiment. The water was pumped through desired cavitating device under controlled conditions. The flow rate was controlled using bypass. The inception of cavitation was confirmed in the pressure drop range of ~ 30 to 50 kPa and 125 to 180 kPa (0.3 to 0.5 bar and 1.25 to 1.8 bar) for vortex diode and orifice respectively from the data of pressure drop vs. flow rate and analyzing the deviation of pressure drop from the usual square law ( $\Delta P$  proportional to square of flow rate or mean velocity) specific to cavitating device. In view of the obtained cavitation inception, the disinfection experiments were carried out at pressure drop conditions of 0.5, 1.0 and 2.0 for vortex diode and for 2, 5 bar for orifice. For the study of oil effect, a known quantity of natural oil was added in the water tank (0.1% or 20 mL for 20 L volume) at the start of the experiment. Samples (10 mL) were withdrawn at regular intervals and colony forming units (CFU) were determined. The reproducibility of the experiments was checked and was found satisfactory.

## **2.6 Disinfection using acoustic cavitation**

Acoustic cavitation was carried out using ultrasound- 40 kHz frequency and 500 W of power (UCP-20 Sonication Unit). A 200 ml of water containing known amount of bacteria was exposed to acoustic cavitation for a period of 15 min. Samples were withdrawn every 5 min and percentage of disinfection estimated. The experiments were performed using both with and without oil addition (Oil addition of 0.1% similar to that in hydrodynamic cavitation)

## **2.7 Disinfection without cavitation**

The contaminated water sample of the same mix of that used for cavitation was separately studied by keeping in incubator shaker at 120 rpm and at 37 °C; with and without oil addition. Samples were withdrawn at regular time intervals and percentage of disinfection estimated.

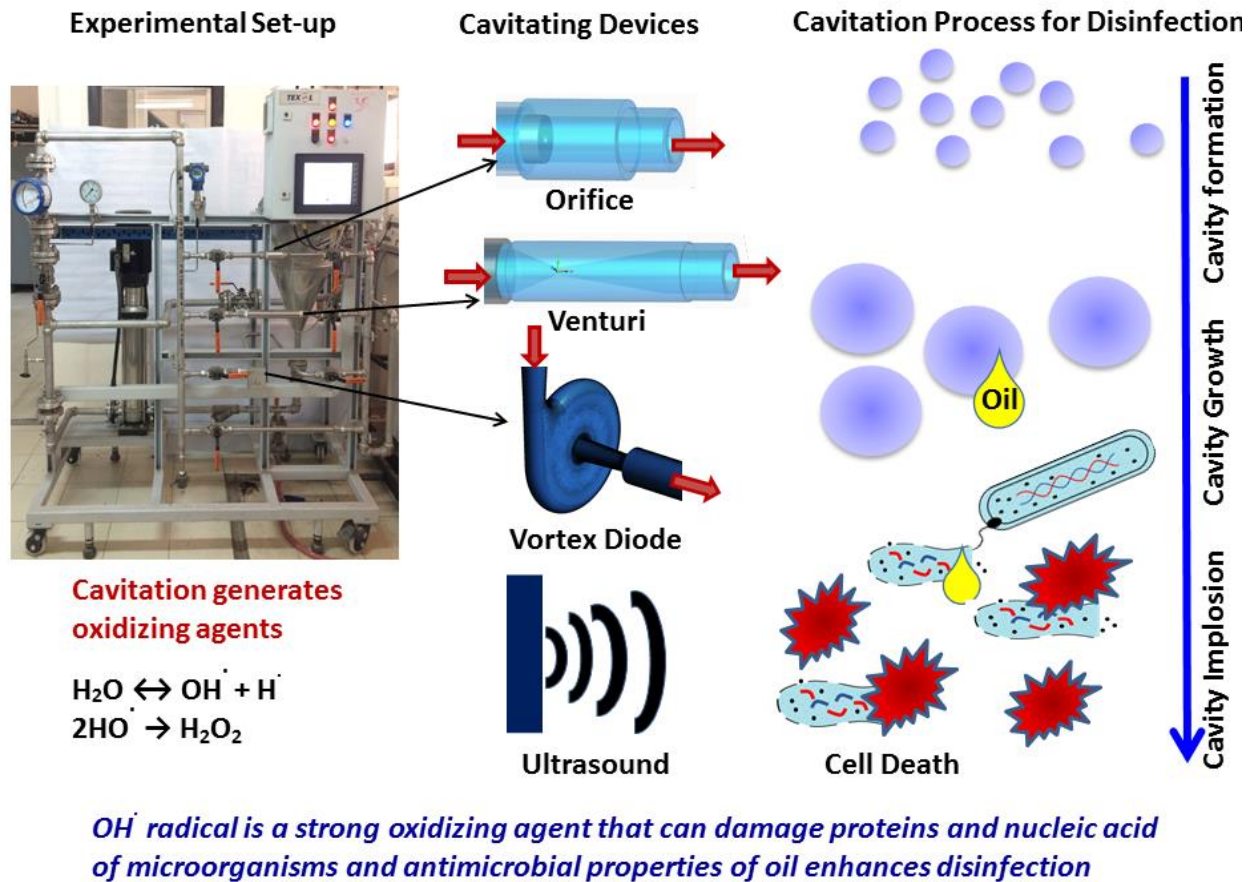


Fig. 1. Experimental set-up and schematic of disinfection using cavitation.

### 3. Results and Discussion

*S.aureus* and *E. coli* were selected as model organisms in the present study to provide proof of concept for application of hydrodynamic cavitation using natural oils for disinfection. *Staphylococcus aureus* is a facultative anaerobic or aerobic gram-positive bacteria having cocci shape, formed in singly, pairs, and irregular clusters and causes variety of skin disease, pustules, septicemia and pneumonitis. *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium mostly occurred in water and known to cause various diseases, including pneumonia, urinary tract infections, and diarrhea. Effect of different process parameters were studied, in isolation as well as in conjunction with natural oil to bring out differences in the cavitation behavior. Effect of different reactor geometry was also evaluated in this regard. The detailed characteristics of natural oils are shown in Table 1.



### 3.1 Effect of pressure drop

Pressure drop ( $\Delta P$ ) is one of the most critical parameters in cavitation reactors as it dictates the number density and quality of the cavities based on the cavitation device type. Apart from quantity and quality of the cavities, the implosion of cavities is most critical to the real oxidation mechanism. Further, high shear generated during the cavity collapse may also physically break open the outer shell of microbes and therefore cause disinfection [28].

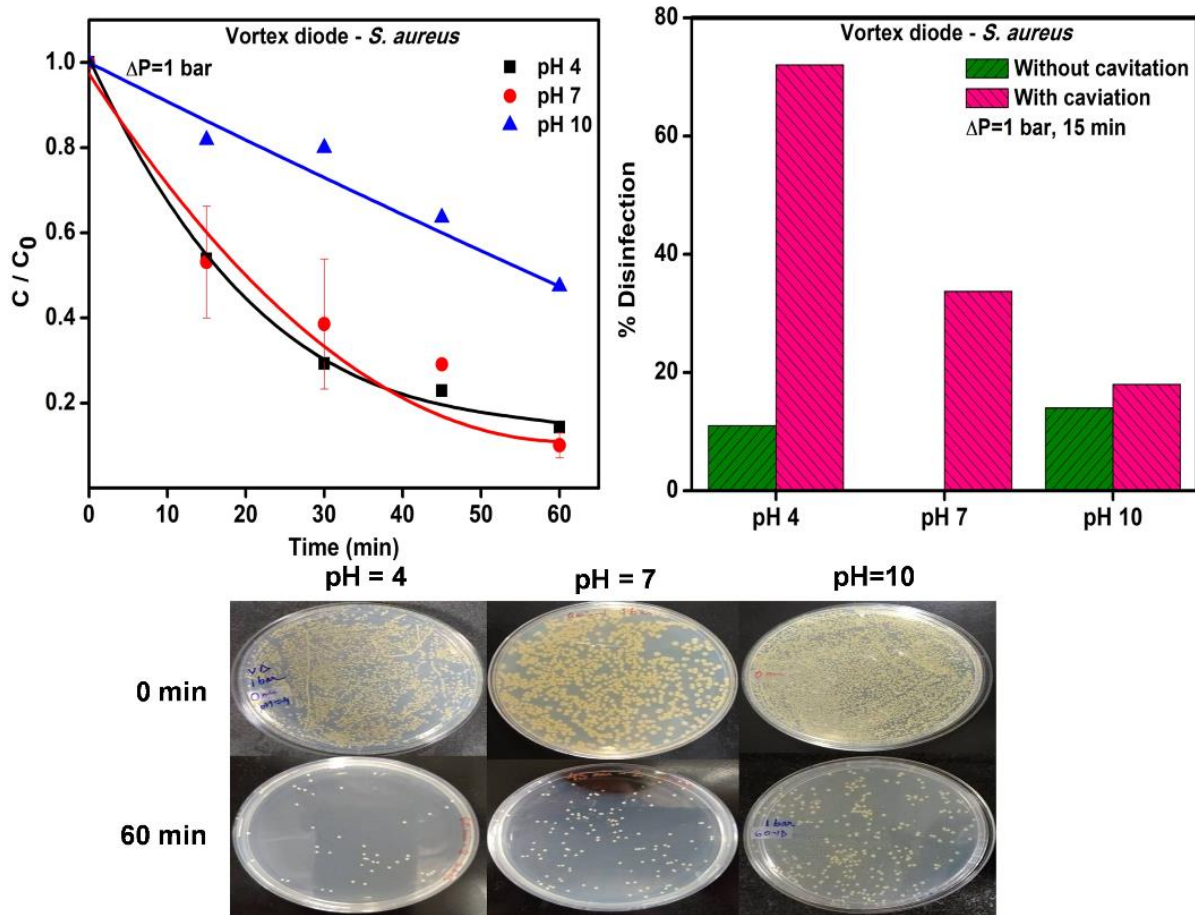
In our earlier studies, the effect of pressure drop for disinfection was discussed in detail and hence, only the essential and new findings in this regard are discussed below [4]. The optimization of pressure drop is utmost important to achieve complete disinfection of microorganism. The results for vortex diode showed that extent of *S.aureus* removal was enhanced from 75 to 89% with increasing pressure drop from 0.5 to 1 bar respectively. Increasing the pressure drop to 2 bar, the *S.aureus* removal efficiency could be further increased to 97 % within one hour, which indicates the consistency and efficacy of vortex diode for disinfection of pathogenic bacteria.

The optimum pressure is typically in between lowest and highest operating pressure corresponding to the low cavity density and cavity cloud respectively [29]. High pressures can also lead to escaping cavities from water without collapse, reducing the production of hydroxyl radicals and therefore reduced disinfection efficiency. Badve et al. [30] also observed increased inlet pressures (i.e. orifice, venturi) leading to increased disinfection up to certain pressure followed by decrease. The present study, however, found consistently increasing disinfection in the case of vortex diode up to 2 bar pressure drop.

### 3.2 Effect of pH on disinfection of *S.aureus*

The effect of pH for disinfection using cavitation has not been investigated so far which could be important from the point of view of wastewater treatment, recycle and reuse. The effect of pH was studied at three different conditions of pH: 4, 7 and 10. All the experiments were conducted at optimized inlet pressure of 1 bar. Fig. 2 shows the results on disinfection of *S. aureus* at different pH with and without cavitation. While acidic conditions favor disinfection, the cavitation is equally effective at neutral pH which is important from its practical application point of view. The acidic pH tends to make microorganisms sensitive to hydrogen ion and enzymatic proteins are affected leading to loss of enzyme catalytic activity and simultaneous denaturation [31]. At pH 4, little disinfection was observed without cavitation, compared to that with cavitation. It is also possible to exploit increased rate of disinfection due to acidity by reducing the treatment time, as 72.0 % disinfection was observed within 15 min, while in the

same time 37 and 20 % of disinfection observed at pH 7.0 and pH 10 respectively. Thus, cavitation, in conjunction with lower pH can have improved disinfection behavior. The enhancement of disinfection at lower pH can be attributed to the lower rate of recombination of hydroxyl radical, thereby making more hydroxyl radicals available for oxidation. It can be presumed that at acidic pH, bacterial enzymes will denature and higher concentration of hydroxyl radicals is possible at interface compared to bulk liquid when the bacteria is in ionic form leading higher percentage of disinfection. The positive effect of acidic pH in cavitation is however largely studied for organic pollutant degradation [17][29][32].

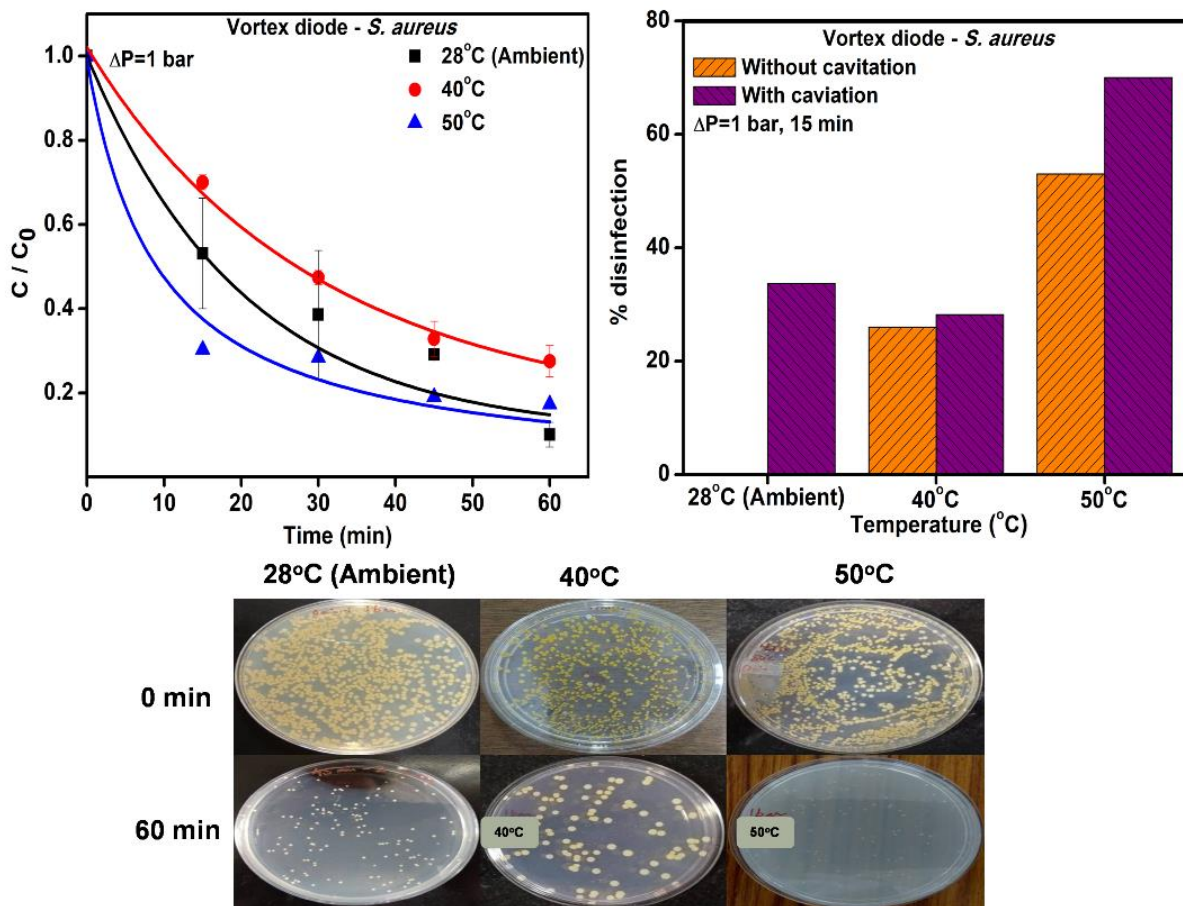


**Fig.2. Effect of pH on water disinfection by hydrodynamic cavitation by vortex diode.**

### 3.3 Effect of temperature on disinfection of *S.aureus*

Temperature plays an important role, for both disinfection and cavitation. Heating to a certain temperature is also one form of disinfection method, though not practical for treating large volumes or isolated small volume treatments. Again, the effect of temperature has been reported in the case of hydrodynamic cavitation for the degradation of organic pollutants, but many systematic studies for disinfection. In the present study, disinfection of *S.aureus* was

investigated using three different temperature viz. 28 (ambient), 40 and 50 °C, with and without cavitation. It can be seen from Fig. 3 that in case of without cavitation, disinfection follows the known trend of increasing efficiency with increased temperature and at 50 °C, about 53 % disinfection could be seen within 15 min and 80.7 % within 60 min as probability of cell death increases [33]. In the case of cavitation, the effect of temperature is marginal, but importantly, the efficacy of cavitation technique is evident at ambient conditions. It may be noted that with increase in the temperature, viscosity, surface tension and gas solubility reduce, thus cavitation intensity and the number of cavity nuclei also can reduce [32]. There are conflicting reports on degradation of pollutants (dyes) with cavitation at increased temperatures indicating both increase and decrease in the rates beyond certain temperature suggesting uncertainties in the cavitation phenomenon at higher temperatures [34,35].



**Fig.3. Effect of temperature on water disinfection.**

### 3.4 Effect of increasing inoculum size

Bacteria concentration in wastewaters can be from insignificant to a very high-  $>10^5$  CFU/mL. The higher concentration of bacteria poses a significant risk to human health and fatal to aquatic life. In the present study, effect of initial bacterial concentration was investigated in the

range 20,000 to 2,00,000 CFU/mL. The results of the effect of concentration are shown in Fig. 4. It is evident that the disinfection percentage remains almost constant, largely in the range 70 - 90 % for increasing inoculum size, which shows reliability and efficacy of cavitation reactor, vortex diode in this particular case, for different concentrations of bacteria. Interestingly, for the effectiveness of the cavitation process, microorganisms should be present in cavitation region so that they get killed by collapsing of cavities in their vicinity [36]. However, for the effect of bacterial concentration, a number of situations may arise such as large number of cavities and less number of microorganism; comparable number of cavities and microorganisms or in another extreme, less number of cavities and largely outnumbered microorganisms. The other uncertain elements of process such as number of cavities collapse or fruitful implosion with deactivation of bacteria, that are not measurable, add to unpredictable behavior of the process. Geometry of the cavitating device is also an important aspect in this regard and vortex diode is found to be much more effective as compared to conventional devices [4,11,18].

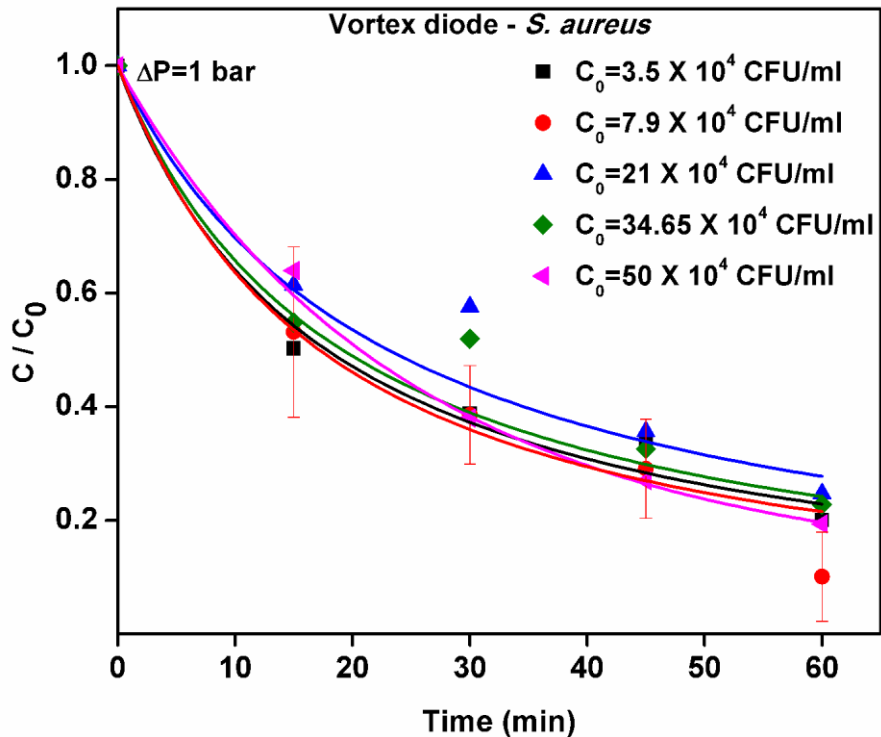


Fig. 4. Effect of inoculum size on water disinfection.

### 3.5 Exploring use of natural oils in cavitation for disinfection

Medicinal plants are rich source of antimicrobial active compound. It would be prudent to exploit the presence of active compounds of natural oils for disinfection of water, without adversely affecting the quality of water. For the first time, the effect of such oils/extracts is being reported for large scale disinfection application and with cavitation. While, such oils can

be easily separated after the treatment, it may also be possible to use positive ingredients from such natural extracts/oils for improving quality of treated water. The properties of some initial selected oils; clove, eucalyptus, cinnamon and castor oil are given in Table-1. The primary basis of selection of these oils for studies in cavitation is that these oils are not harmful in nature. The effect has been evaluated using different cavitating devices i.e. vortex diode, orifice and also for acoustic cavitation.

The results of disinfection are given in Fig. 5 for the four oils and for vortex diode as a cavitating device; for clove oil and orifice as a cavitating device. The proof of concept is also provided for acoustic cavitation and clove oil in Fig. 5c. Only a fraction of oil, 0.1% of total volume of water was added in all the experiments. There are a number of observations from these results (Fig. 5 and Table-2). First, the oil can have both positive and negative impact in terms of extent of disinfection:

1. Clove oil and eucalyptus oil have best effect in terms of increased efficiency while castor and cinnamon oils have lower disinfection efficiency compared to hydrodynamic cavitation alone for vortex diode as a cavitating device.
2. A very important aspect of the results of Fig. 5a, is that there is an order of magnitude increase in the rates of disinfection, implying significantly reduced time of operation. For example, in the case of clove oil and eucalyptus oil, the increase in the rates in initial period is as high as 2-4 folds in 15-20 minutes compared to cavitation alone.
3. The results clearly indicate positive impact of clove and eucalyptus oil, both in terms of rate of disinfection as well as for increased disinfection.
4. Similar results are observed in case of orifice using a pressure drop of 2 bar (Fig. 5b). It is to be seen that orifice requires significantly higher pressures, almost double or more, as compared to vortex diode for similar extent of disinfection. In 15 minutes, ~ 32 % disinfection of *S.aureus* was observed by orifice, which was enhanced to 55 % by addition of clove oil.
5. The concept of cavitation using oil for disinfection was validated using acoustic cavitation as well and from the results of Fig. 5c it is evident that acoustic cavitation using oil (0.1%) has hugely increased the disinfection; without oil and with acoustic cavitation alone, there was negligible disinfection. Use of clove oil with acoustic cavitation here gave ~51 % disinfection in 15 minutes.
6. For the case of cinnamon oil and castor oil, the disinfection rates were adversely affected.
7. It is evident that selection of natural oils is crucial for improving the rates and extent of disinfection that could also positively impact in reducing the cost of operation.

**Table 1. Properties of natural oils used in present study**

Sr. No.	Name of oil	Viscosity (poise)	Surface tension (dyne/cm)	Active ingredient (%)	Reference
1	Clove	0.066 ± 0.006	5.8 ± 0.72	Eugenol (83.13%)	[37]
2	Eucalyptus	0.337 ± 0.033	7.33 ± 1.49	1,8-eucalyptol (72.71)	[37]
3	Cinnamon	0.041 ± 0.001	23.04 ± 0.07	Cinnamaldehyde (82.5%), eugenol (0.5%)	[37]
4	Castor	6-8	39	Ricinoleic acid (85-95%)	<a href="https://www.drugfuture.com/chemdata/castor-oil.html">https://www.drugfuture.com/chemdata/castor-oil.html</a>

It is quite instructive to evaluate the differences in the disinfection behaviour of different oils, especially from the view point of cavitation where bubbles/cavities get formed, grow and finally collapse to yield desired impact. Three factors can directly affect the collapse or implosion of cavities- the surface tension of the bubble, the inertia of the fluid and the pressure of the gas inside the cavities [38]. The pressure difference between the inside and outside of a cavity depends upon the surface tension and the size of the cavity. Thus, the properties of oil can modify the cavitation behaviour according to their physical properties. Apart from the physics of the bubbles and their altered collapse, antibacterial activity of the natural oil is another important aspect. A high antibacterial activity of *eucalyptous* oil reported by Lu et al. [39] and Bachir and Benali, [25]. The high disinfection ability of *eucalyptous* oil is due to the presence of 1,8-cineole active compound. The active compound of eucalyptus oil can destroy the permeability of bacterial membranes, leading to loss of electrolytes such as  $K^+$ ,  $Na^+$  and  $Ca^{+2}$  [39]. Similarly, clove oil has high bactericidal activity due to high level of eugenol [26]. The eugenol can react with the phospholipids of the cell membrane altering its permeability and as a consequence denature cell protein. The denaturation of cell protein causes death to cell [40]. The lower disinfection using *cinnamon* (62%) and castor oil (60%) may be attributed to adverse effect on number and quality of the cavities, as both the formation and energy release rate depends on surface tension and viscosity of oil. Increasing surface tension reduces size of the cavities and promotes less violent collapse [41]. Thus, in the present case, lower disinfection is observed for oils having high surface tension properties (castor oil 39 dyne/cm, cinnamon 23.04 dyne/cm) compared to oils having low surface tension (eucalyptus oil 7.3 dyne/cm, clove oil 5.8 dyne/cm). Further, microbial inactivation is process in which viability of organisms exposed to oil additive varies with time [39]. The inactivation depends on the type of microorganism, type



and concentration of oil additive, and environmental conditions such as temperature and pH. Thus, the effect of oil can be significantly different for different microorganisms. However, these aspects are complex in nature due to interacting physical, chemical and microbial attributes and hence require more detailed investigations.

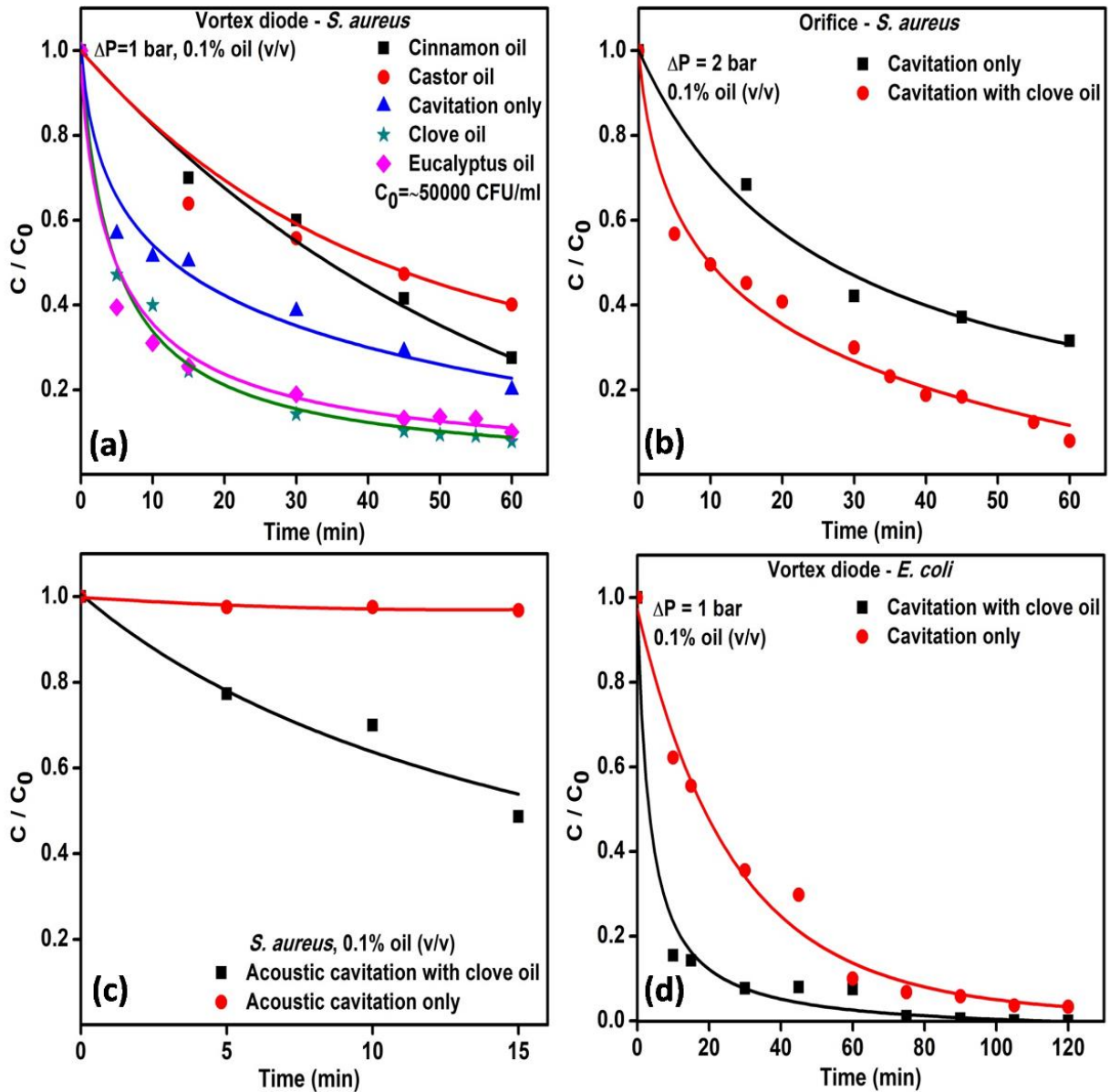


Fig.5. Effect of oil in cavitation for disinfection.

(a) Vortex diode – *S. aureus* (b) Orifice- *S. aureus* (c) Acoustic cavitation- *S. aureus* (d) Vortex diode- *E. coli*

The methodology was extended for the removal of gram-negative bacteria (*E. coli*) and only the proof of concept is provided by using clove oil and vortex diode as a cavitating device using the optimum pressure drop of 1 bar. The results are shown in Fig. 5d which clearly confirm order of

magnitude increase in the initial rates of disinfection compared to cavitation alone and practically complete removal can be obtained in 90-100 min.

### 3.6 Kinetics of disinfection

A development of model based on per-pass conversion for hydrodynamic cavitation was presented in our earlier work [4] and the same is extended to evaluate kinetic data of the present study under different conditions. The cavitation process can be schematically shown as per Fig. 6.

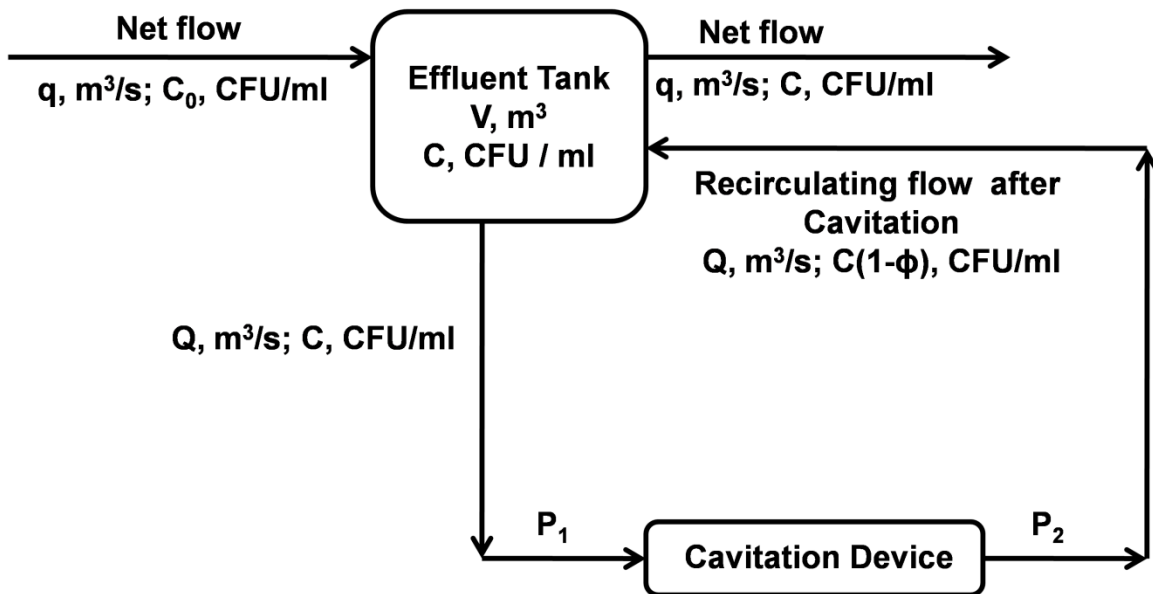


Fig. 6. Schematic diagram for decontamination of water using cavitation.

The kinetics of disinfection can be modelled in two ways: one is by conventional rate model and another is cavitation based model [42]. It was observed that the concentration of bacteria decreased exponentially with time and the kinetic data for disinfection of water can be fitted using Pseudo-first order equation in terms of  $k_G$  (growth rate of microorganisms) and  $k$  (disinfection rate of microorganisms).



Step	Conventional rate model	Cavitation based model
1	Assuming first order reaction $-\frac{dc}{dt} = kC$ k is a rate constant	Pseudo-first order equation in terms of $k_G$ (growth rate of microorganisms) , k (disinfection rate) $V \frac{dc}{dt} = V (k_G - k)C$
2	Integration of rate gives the pseudo first-order relationship $\ln \frac{C}{C_0} = -kt$ $C = C_0 e^{-kt}$	The concentration of microorganisms C can be obtained by assuming the rates to be constant: $C = C_0 e^{-(k-k_G)t}$ where $C_0$ is the initial concentration of bacteria (CFU/ml), t is the time for disinfection (min)
3	Not Applicable	The extent of disinfection in terms of per-pass disinfection factor ( $\phi$ ) and number of passes (n): $V \frac{dC}{dt} = V k_G C - Q \phi C ; n = \frac{Q}{V} t$
4	Not Applicable	For the assumption of constant $\phi$ and $k_G$ , the concentration of microorganism at time t is estimated for the residence time of $\tau$ (V/Q). $C = C_0 e^{-(\phi - k_G \tau)n}$
5	$C = C_0 e^{-kt}$	Correlating the growth rate constant to doubling time, $t_D$ as $k_G = (\ln 2/t_D)$ and assuming negligible growth of microorganism during the treatment time, the disinfection of water can be described as: $C = C_0 e^{-kt} = C_0 e^{-\phi n}$
6	The effective disinfection rate constant (k) is: $k = \frac{\ln(C_0/C)}{t}$	The effective disinfection rate constant (k) may be related to residence time ( $\tau$ ) and $\phi$ as: $k = \frac{\phi n}{t} = \frac{\phi}{\tau}$
7	The overall cavitation yield, $Y = \frac{V(C_0 - C)}{\Delta P Q t} \text{ CFU/J}$	The overall cavitation yield, can be obtained as: $Y = \frac{V(C_0 - C)}{\Delta P Q t} \text{ CFU/J}$
8	Not Applicable	For small values of $\phi$ , $Y = \frac{\phi C}{\Delta P} \text{ CFU/J}$
9	Rate of disinfection, R is: $R = kC_{avg} \text{ CFU/(ml s)}$ $C_{avg} = \frac{C_0 + C}{2}$ $C_{avg}$ is average concentration;	The average disinfection rate over time 't <sub>op</sub> ' is: $R_{avg} = \frac{C_0(1 - e^{-kt})}{t_{op}} = \frac{C_0(1 - e^{-\phi n})}{t_{op}} \text{ CFU/(ml s)}$

The mathematical model based on the physical description of cavitation process is closer to the real life operation and is also easy to solve using the experimentally obtained parameters such as flow rate (Q), volume (V) and concentration-time data. The value of number of passes is of practical importance since it determines the cost of operation and lower values are desirable. The value of per-pass disinfection factor can be simply obtained using Eq. 6. As discussed in our earlier work, per-pass disinfection factor based cavitation model is more realistic, as nature of geometry of the cavitation device can be reflected into per-pass disinfection. The mathematical treatment clearly indicates dependence of disinfection on residence time and less dependence on initial concentration compared to conventional reaction rate model.

The results of kinetics study for the conventional rate model and those using cavitation model are presented in Table 2, specifically for 15 minutes of operation, where differences in the initial rates are evident. Similar to our earlier findings [4], the values of rate of disinfection are very high indicating effectiveness of cavitation, in general and vortex diode, in particular. Thus, the results clearly establish strong dependence of rate of disinfection upon type of cavitating device. In this work, apart from different cavitating devices, we have also attempted to evaluate the performance under acoustic cavitation (using ultrasonic bath) and the rates of disinfection for the same are also listed in Table-2. Further, oil as an additive was evaluated for the improvement of the process. A number of interesting observations could be made in this regard.

1. The rates of disinfection in hydrodynamic cavitation are significantly/ order-of-magnitude higher compared to acoustic cavitation.
2. The rates of disinfection are higher using vortex diode as compared to orifice and orifice requires significantly higher pressures compared to vortex diode.
3. Low pH (4) gives substantially increased rates of disinfection.
4. Higher temperature, naturally and as expected, improves disinfection.
5. There is drastic effect of oil on disinfection behavior. While the two oils, namely clove and eucalyptus oil positively alter the cavitation behavior, the other two oils, cinnamon and castor oils adversely impact the disinfection behavior. This effect is also observed even in the case of acoustic cavitation, where more than 20 times increase in the initial rate of disinfection was observed by addition of clove oil.
6. The reasons for the variations in different oils can be explained on the basis of characteristics of oil and the constituent properties, as explained in the earlier section and also using the proposed mechanism.
7. Vortex diode is found to be superior compared to orifice even in the case of increased rates of disinfection by oil addition.

The differences in the rates for acoustic cavitation and hydrodynamic cavitation are quite understandable on the basis of the intensity of cavitation since large differences have been reported even within acoustic cavitation using ultrasonic bath and ultrasonic horn [14]. The temperatures also aid disinfection process and increased rates can be obtained at higher temperatures [30]. The conventional methodologies typically considered using the combination of different treatment methods. The oil as an additive can be one alternative to many of the hybrid techniques reported in the literature such as hydrogen peroxide treatment, ozone, ultrasonic cavitation coupled hybrid processes; implying uniform and intense cavitation effects in disinfection with possible health benefits for the oil characteristics apart from the conventional attributes of high efficiency and also ease of operation compared to ultrasonic cavitation, conventional hydrodynamic cavitation or hybrid methods.

The effect of temperature on per-pass disinfection factors for *S. aureus* is shown in Fig. 7. For vortex diode and under ambient conditions, as the pressure increases from 0.5 bar to 2 bar, per-pass disinfection factors increase from 0.057 to 0.086 and subsequently energy dissipation rate also increases. However, similar trend is not found at higher temperatures (40°C and 50°C), as the pressure increase reduces per-pass disinfection factor partly due to the vapour cloud formation; large number of cavities lead to large vaporous bubbles which get carry-forward without collapsing [43, 44].

The impact of addition of natural oils on per-pass disinfection, especially in the initial rates, is shown in Fig. 8 for vortex diode at the optimum pressure drop of 1 bar. The errors were largely below 1.5% for all sets, except for eucalyptus oil. The maximum error for eucalyptus oil was ~8%. It can be seen that per-pass disinfection factors for *S. aureus* are higher for clove and eucalyptus oil with cavitation than other oils. It is evident that in 15 min, cavitation with clove oil and cavitation with eucalyptus oil showed more than 100% increase in the per-pass disinfection as compared to cavitation alone. The benefits diminish with time and hence selection of best suitable oil and time of treatment is crucial. Thus, the results clearly highlight that the increased rates of disinfection and per-pass disinfection in hydrodynamic cavitation can be exploited to reduce the time of operation, consequently to reduce the cost of disinfection.

Although the initial rate of disinfection could be significantly increased using oil as an additive, more rigorous efforts are further required to establish the relationship of oil properties and its synergy with cavitation process so that appropriate guidelines can be developed for the selection of most suitable oil.

**Table 2: Rate of disinfection for *S.aureus* (15 min)**

Study	Parameter	Initial CFU/ml	% Disinfection	Rate of disinfection, CFU/(mL.s) (conventional model)	Rate of disinfection, CFU/(mL.s) (cavitation model)	Rate constant (s <sup>-1</sup> )×10 <sup>4</sup>	Per-pass disinfection (φ)
Effect of Pressure Vortex Diode	<b>ΔP, bar</b>	50000	32	18.0	17.8	4.3	0.0598
	0.5						
	1						
Effect of pH Vortex Diode (ΔP=1 bar)	<b>pH</b>	78500	71	70.5	62.4	14.0	0.1314
	4						
	7						
Effect of Temperature, (°C) Vortex Diode (ΔP=1 bar)	<b>T, °C</b>	68000	21	15.6	15.6	2.6	0.0249
	28						
	40						
Effect of oil 0.1% (v/V) Vortex Diode (ΔP=1 bar)	<b>Oil</b>	35000	63	26.6	24.6	11.1	0.1106
	Clove						
	Eucalyptus						
	Cinnamon						
Effect of Pressure Orifice (ΔP=2 bar)	<b>Oil</b>	43800	74	41.7	36.2	15.2	0.1491
	Clove						
	Eucalyptus						
	Cinnamon						
Effect of oil 0.1% (v/V) Orifice (ΔP=2 bar)	<b>Oil</b>	58000	30	19.5	19.3	4.0	0.0400
	Clove						
	Eucalyptus						
	Cinnamon						
Effect of oil 0.1% (v/V) Acoustic (15 min)	<b>Oil</b>	73500	36	29.9	29.4	5.0	0.0497
	Clove						
	Eucalyptus						
	Cinnamon						
Effect of Pressure Orifice (ΔP=2 bar)	<b>ΔP, 2 bar</b>	38000	32	13.5	13.3	4.2	0.0970
Effect of oil 0.1% (v/V) Orifice (ΔP=2 bar)	<b>Oil</b>	25000	55	16.0	15.2	8.8	0.2061
	Clove Oil						
Acoustic (15 min)		66100	3.18	2.3	NA	0.36	NA
Effect of oil 0.1% (v/V) Acoustic (15 min)	<b>Oil</b>	62700	51	37.3	NA	8.0	NA
	Clove Oil						

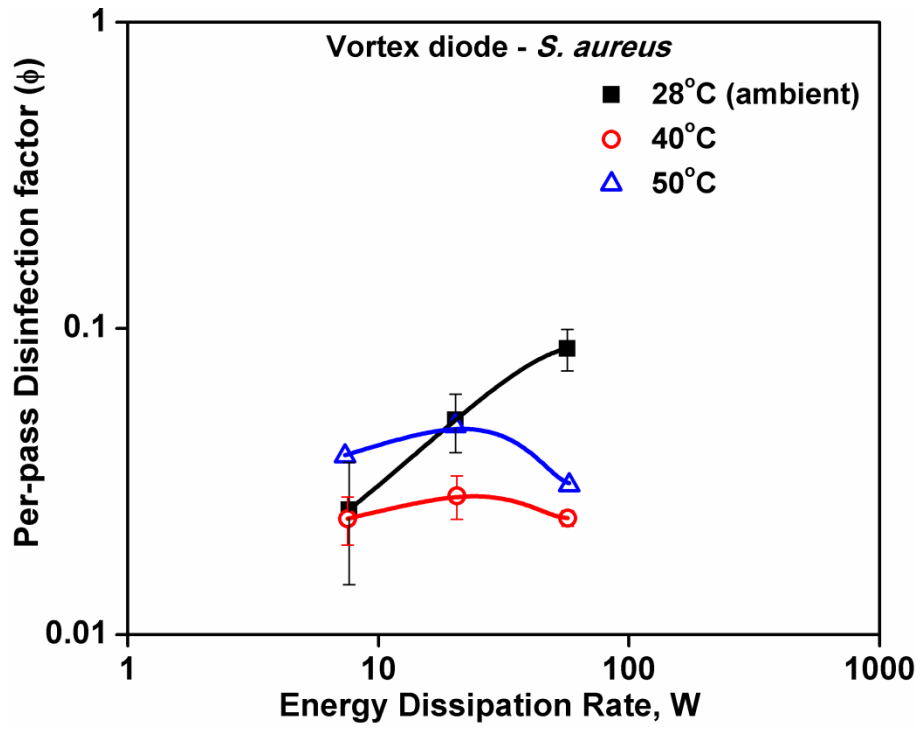


Fig. 7. Effect of Temperature- Per- pass disinfection factors at 60 min.

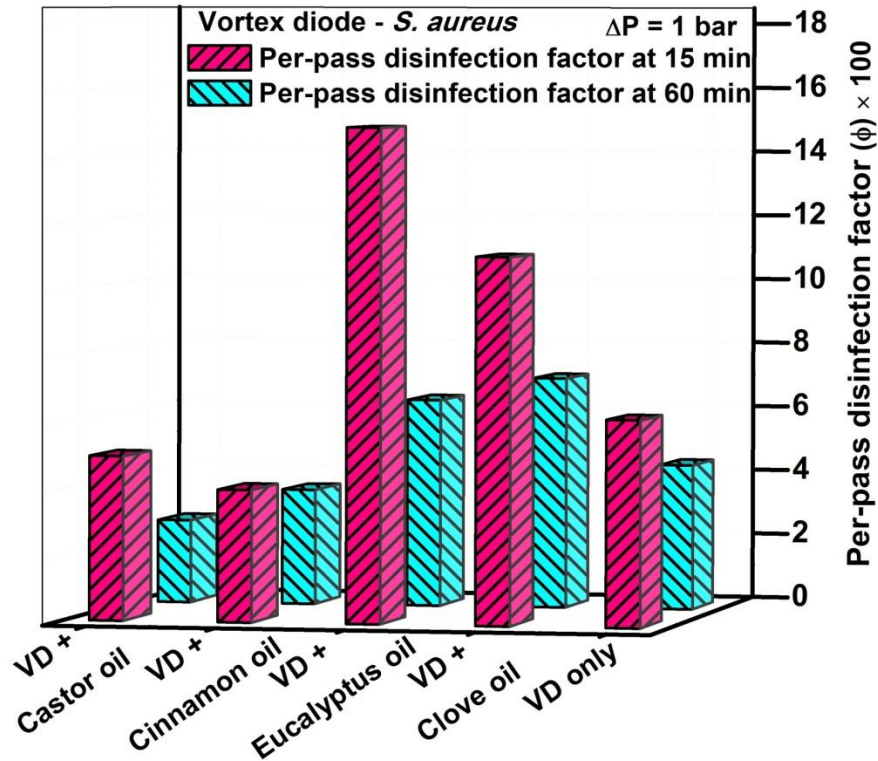


Fig. 8. Effect of oil on per- pass disinfection (VD-Vortex diode)

### 3.7 Disinfection of *S.aureus* from water using hydrodynamic cavitation: A plausible mechanism

Oxidation process is known since long time for the destruction of bacteria, however their usage is limited due to major shortfalls. The conventional oxidation is a slow process and sometimes required additional catalyst to improve the removal rate. Also, the treatment is unsatisfactory for the complete killing of bacteria and rather only deactivates the bacteria. As per the EPA manual of water treatment, hydrogen peroxide is considered as a poor disinfectant. Thus, it would be prudent to find alternatives to existing many chemical disinfectants in this regard.

In the present study, cavitation with addition of natural oil was shown to achieve complete destruction of bacteria within shorter time period. The selection of natural oils is, however, not straightforward and a number of parameters are to be considered that would directly impact either the disinfection process or the cavitation process. Thus, in order to obtain synergetic effect of both cavitation and anti-microbial properties of natural oils, it is essential to envisage the possible mechanism of disinfection. Based on the results of the present study, a plausible mechanism for cell destruction is proposed and shown in Fig. 9. The destruction of cells can be attributed to hydroxyl radicals generated during the cavitation resulting into oxidative damage

of the cells. Localized heat conditions of cavity implosion can also damage the DNA. The main reason for cell death is cell disruption since once cell membrane and/or cell wall is severely damaged, the bacteria become dead without DNA and protein denaturation. It has been well reported that important constituents of microorganisms such as proteins, lipids, DNA and polysaccharides can be affected by oxidation. The reactive species such as  $\bullet\text{OH}$  radicals can attack and oxidise double bonds and sulfhydryl groups of protein constituents and produce oxidative stress that creates unalterable consequences for the microorganisms (Zupanc et al., 2019; Gashchin and Viten'ko, 2018). Moreover, the active compound present in the natural oil can react with the phospholipids of the cell membrane thereby altering its permeability and also denature cell protein. The denaturation of cell protein causes cell death [40].

To further support the mechanism, the difference in cell morphology of bacterial cell before and after the cavitation with oil treatment was investigated using SEM and TEM techniques (Fig. 10). The results of FE-SEM clearly highlight the intact nature of the cells of *S.aureus* (0 min), round shaped and in a cluster form before treatment (Fig. 10a) and their mutilated form after the treatment (60 min), where cellular morphology of bacteria is changed in terms of increased cell permeability, distorted cell membrane and creation of holes or wrinkles on bacteria (Fig. 10b). These results were further reinforced by TEM analysis. The results of TEM analysis confirmed that at 0 min, the bacterial cell was intact as well as round shaped with clear cell membranes (Fig. 10c), while after combined cavitation with oil process, the cells were seen unevenly distributed, condensed, loss of cytoplasm and leakage of cytoplasmic content (Fig. 10d). Lu et al. [39] studied on antimicrobial activity of eucalyptus oil towards *Pseudomonas* sp., and stated that the antimicrobial effect of eucalyptus oil may be due to the active ingredient of oil that reacts with surface of bacterial cell and penetrate to plasma membrane leading to distortion of bacterial cell. The morphological changes and alteration of bacteria after oil treatment have been reported by several researchers [20, 39].

While, the antibacterial activity of various oils and reasons thereof are well reported in the literature, its synergetic effect in enhancing efficiency in combination with other processes such as cavitation are not well researched so far. The plausible mechanism as depicted in Fig. 9 therefore is an attempt to qualitatively provide causes of cell damages, such as the membrane rupture due to extremely high stress produced during cavitation process, generation of extremely high temperatures during bubble implosion leading to the leakage of cytoplasmic matter apart from possible cell membrane rupture and DNA damage due to generation of active free radicals. The role of cavitation in disinfection in this regard is in accordance with that reported by Cerecedo et al. [20]. Palacios et al. [45] have reported *B. stearothermophilus* elimination by ultrasonic treatment and proposed that high pressures affected the permeability of protoplast membrane due to which dipicolinic acid, calcium and other low molecular weight

substances get leaked and cell properties get modified which is in agreement with the present findings.

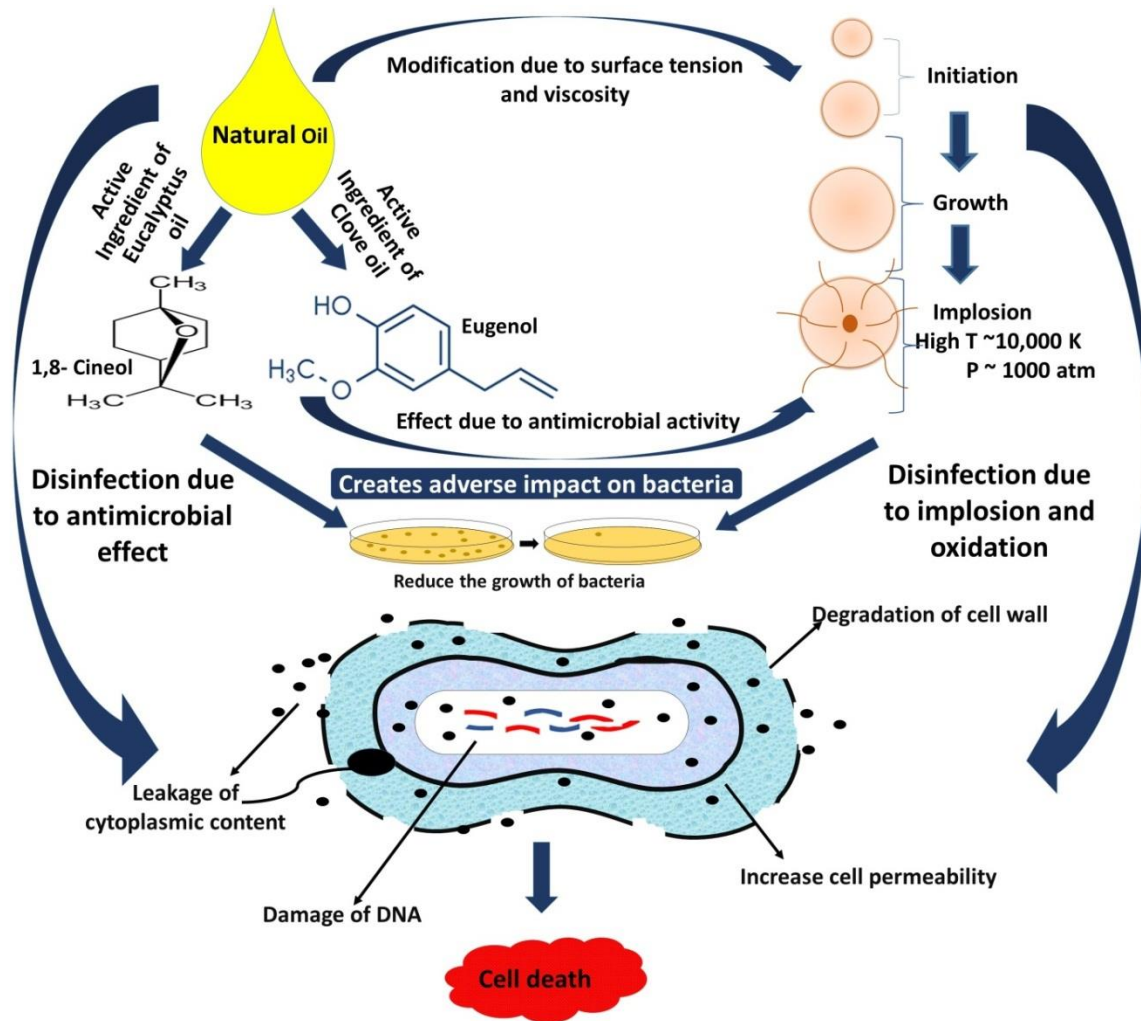
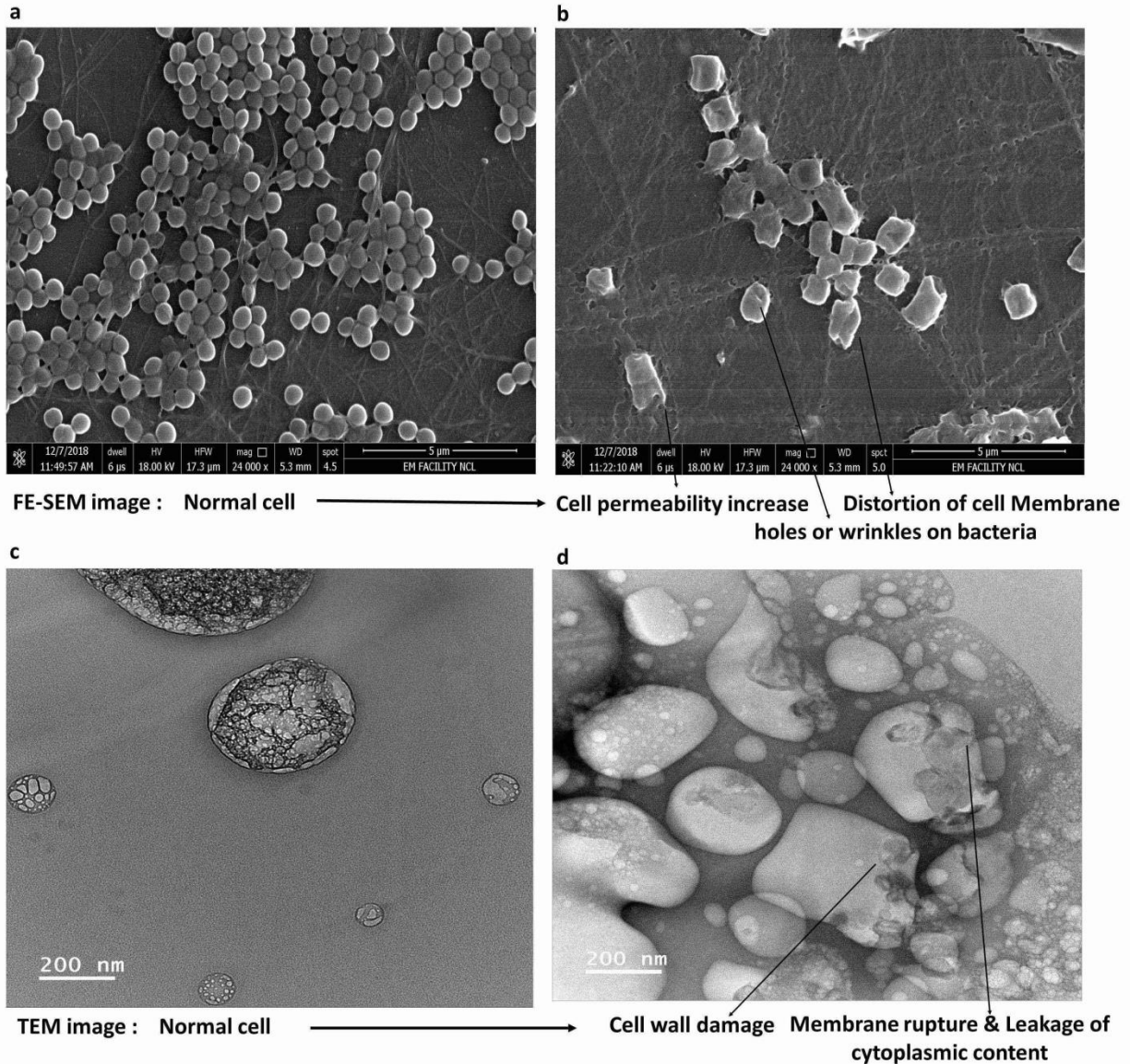


Fig. 9. Plausible mechanism of disinfection using natural oils and cavitation.





**Fig. 10. Effects of cavitation with natural oil on morphology of bacteria.**

**FE-SEM image (24,000 X) (a) 0 min sample (b) 60 min of treatment (c) TEM image, 0 min (d) TEM image, 60 min of treatment**

### **3.8 Comments on the practical application of the newer method**

The cavitation process for disinfection of water is known and has been well reported in the literature, especially for acoustic cavitation and for hydrodynamic cavitation using conventional devices such as orifice, with and without process intensifications such as ozone, hydrogen peroxide addition, and so on. The success with these was, however, limited and as a result,

practical application of such techniques is not available for either household applications or for large scale water treatment installations.

The results presented in this work are important from two counts. One, the hydrodynamic cavitation can eliminate the bacteria to the extent of 100% as desired by various norms. Secondly, the proof of concept in the form of addition of natural oils can significantly increase the rate of disinfection, thereby reducing the time of operation and consequently reducing the cost of operation. Although, the natural oil can be separated after use using standard methods, it may also be possible to exploit the health benefits of specific oils, with appropriate designs. Further, in such cases, it may also be useful to couple the process with other established methods such as adsorption for complete removal of bacterial contamination for cost optimization. It is necessary to investigate the effect of natural oils in detail to further enhance the performance of the developed process.

A typical flow diagram for the practical operation and conceived prototype design is shown in Fig. 11. The process essentially has the following essential steps:

1. Creating a two phase system by contacting of oil phase with water/aqueous phase;
2. Creating of third vapor phase and conditions of in situ cavitation;
3. Allowing cavities to collapse so as to generate in situ hydroxyl radicals or hydrogen peroxide;
4. Allowing removal of bacteria from the contaminated water containing bacteria such as *E. coli*, *S. aureus* etc.
5. Separating the oil phase and aqueous phase after the process is completed;
6. Removing bacteria or having bacteria content altered as per desired limits
7. Recycling oil for further use.

In the case of partial destruction of bacteria using cavitation, the process can be suitable combined with other established such as adsorption, where suitable adsorbents effective for disinfection can be employed, e.g. silver nanocomposites, bio-nanocomposites and so on [8, 9]. It is believed that process integration using hydrodynamic cavitation and cavitating device-vortex diode has a potential to provide practical solution to disinfection of water so that the disadvantages of existing chemical disinfection processes can be circumvented.

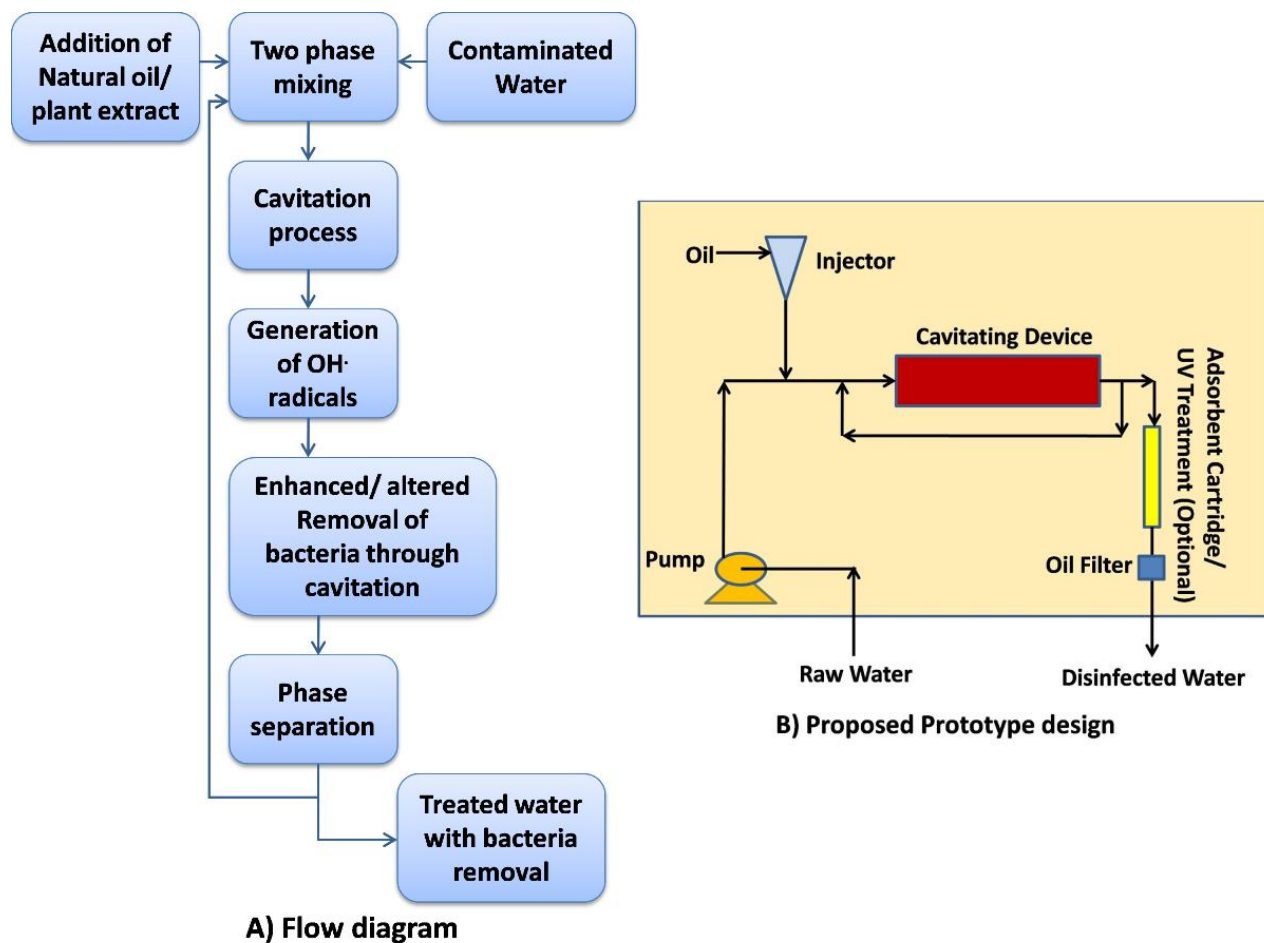


Fig. 11. Flow diagram for disinfection using cavitation and proposed prototype.

## Conclusions

The present study, for the first time, clearly demonstrated the useful application of natural oils having antibacterial properties in combination with cavitation technology to enhance the efficiency of disinfection of water. Both gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria can be effectively removed by optimizing the process parameters for hydrodynamic cavitation such as pressure, temperature and by use of suitable oil. It was shown that a very small percentage of oil (0.1%) can be sufficient to enhance initial rates of disinfection to the extent of 2 times for *S. aureus*, 5 times for removal of *E. coli* in the case of hydrodynamic cavitation using vortex diode and up to 17 times as compared to that in acoustic cavitation. The nature of oil modifies the cavitation behavior and hence a number of possible combinations can be anticipated in this manner. In the present study, clove oil was found to be the most effective natural oil in combination with cavitation as compared to eucalyptus, cinnamon and castor oil.

Further, process is effective even at very high concentrations of bacteria, not reported so far. Practically 100% disinfection can be obtained using the hydrodynamic cavitation technology at very low pressure drop conditions, as low as 1 bar, especially for vortex diode as a cavitating device compared to orifice. A possible mechanism is proposed for the effect of oil and hydrodynamic cavitation in cell destruction through the rupture of cell wall, oxidative damage and possible DNA denaturation. The increased rates of disinfection using oils, where natural oils can be perceived as biocatalysts, along with reduced time and ease of operation, easy scale-up indicate potential to provide significant advantages in practical applications.

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## **Conflict of Interest**

One of the authors (VVR) is a founder director of Vivira Process Technologies Pvt. Ltd. which commercially offers vortex diode based cavitation devices.

## **Nomenclature**

C	Concentration, CFU/ml
$C_0$	Initial concentration, CFU/ml
k	Disinfection rate constant
$k_G$	Growth rate constant
n	Number of passes
P	Pressure, bar
$\Delta P$	Pressure drop, bar
Q, q	Flow rate, m <sup>3</sup> /s
t	Time, s
V	Volume, Liters
$\phi$	Per-pass disinfection factor
$\tau$	Residence time, s

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