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Effects of cavitation on different microorganisms: The current understanding of the mechanisms taking place behind the phenomenon. A review and proposals for further research

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ABSTRACT

A sudden decrease in pressure triggers the formation of vapour and gas bubbles inside a liquid medium (also called cavitation). This leads to many (key) engineering problems: material loss, noise, and vibration of hydraulic machinery. On the other hand, cavitation is a potentially useful phenomenon: the extreme conditions are increasingly used for a wide variety of applications such as surface cleaning, enhanced chemistry, and wastewater treatment (bacteria eradication and virus inactivation).

Despite this significant progress, a large gap persists between the understanding of the mechanisms that contribute to the effects of cavitation and its application. Although engineers are already commercializing devices that employ cavitation, we are still not able to answer the fundamental question: What precisely are the mechanisms how bubbles can clean, disinfect, kill bacteria and enhance chemical activity?

The present paper is a thorough review of the recent (from 2005 onward) work done in the fields of cavitation-assisted microorganism's destruction and aims to serve as a foundation to build on in the next years.

1. Introduction

The research on the potential of cavitation exploitation is currently an extremely interesting topic. Availability of water is becoming an increasing concern in the globalized world, in both developed and developing countries. Therefore, an efficient and clean disinfection technology, such as optimised employment of cavitation, would be readily welcome to substitute or be combined with the existing ones.

1.1. Problem identification

Due to escalating pollution, the world's clean water supplies are becoming seriously endangered and for a lot of countries, clean water is a luxury that cannot be taken for granted anymore. Therefore, implementing wastewater (WW) recycling and assuring impeccable drinking water sources are becoming more and more important. On one hand, WW effluents contain pathogenic microorganisms like bacteria (i.e. from genera *Vibrio*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*) [1] and enteric viruses (i.e. noroviruses and caliciviruses) [2], which can both cause serious infections in organisms that encounter the contaminated water. On the other hand, the main problem for drinking water supply systems is contamination by algae and cyanobacteria. They can cause algal blooms that are not problematic only because they affect the whole water ecosystem but also because of the toxins they release. These toxins are dangerous for many receiving organisms, humans included [3]. To ensure the safe reuse of WW and the use of drinking water, disinfection is an imperative step in the water treatment scheme.

Increasing world's population also leads to augmented food consumption. Due to this more and more attention is given to the production of high-quality food. This means that food preservation, in terms of nutritional, sensory, ensured bioactivity and microbiological

Abbreviations: AC, acoustic cavitation; BT, blow through cavitation device; CFU, colony forming units; EOM, extracellular organic matter; HC, hydrodynamic cavitation; HFUS, high frequency ultrasound; LFUS, low frequency ultrasound; LPS, lipopolysaccharides; PC, pump + constriction cavitation device; PFU, plaque forming units; ROS, reactive oxygen species; RS, rotor-stator cavitation device; SEM, scanning electron microscopy; TEM, transmission electron microscopy; CFU, colony forming units; US, ultrasound; VBNC, viable but not culturable; WW, wastewater

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aspect, is of utmost importance [4]. The most important culprits of food spoilage are bacteria, viruses, yeasts and moulds [5].

Another consequence of global population's growth is a more rapid depletion of fossil fuels and fast development of fields like biotechnology, pharmacology and food industry. In order to match the increasing energy demand and to allow these industries to develop further, more effort should be directed into research of novel renewable energy sources and into the search of new and natural sources of compounds. Various types of microorganisms seem to fit both requirements. Algae species from genera like *Nannochloropsis*, *Chlorococcum*, *Scenedesmus*, and *Tetraselmis* are being extensively studied for extraction of lipids to produce biofuels [6]. Similarly, extraction of bioactive compounds like pigments, proteins, antioxidants, lipids, and polysaccharides from yeast (i.e. *Saccharomyces* sp. and *Kluveromyces* sp.) and algae species from genera (i.e. *Dunalliela*, *Chlamydomonas* and *Chlorella*) is also gaining more and more attention [7,8].

Despite completely different final goals, disinfection of water, food preservation and the use of microorganisms for extraction, have one thing in common: Destruction of microorganisms!

1.2. Existing methods for microorganism's destruction

Different mechanical and non-mechanical methods for destruction of various types of microorganism have been extensively researched and reported in the literature [8–13] Commonly used methods for water disinfection include processes, such as chlorination, ozonation, and UV irradiation. These methods are effective to some degree, but they unfortunately also have disadvantages. UV irradiation causes reversible damage to bacteria's DNA [14] and is not very effective when microorganisms are packed into flocs [15]. Chemical disinfection methods like chlorination can result in the formation of by-products [16] and lead to secondary pollution [17,18]. Special attention is also needed when dealing with algae blooms, since using these methods can lead to a release of a toxic compound microcystin [19]. Similarly, thermal pasteurization is still extensively used for food preservation, yet it is not appropriate for all types of food and can result in unwanted effects that impact food's nutritional content and quality [20].

Due to the above-mentioned unwanted side-effects of classical disinfection and destruction methods, the search for alternative, effective, environment-friendly and economical methods with less unwanted effects is increasing. One such promising method is cavitation.

1.3. The need for a thorough review

The fields where the introduction of cavitation is considered as a solution are many – pharmacy, chemistry, cleaning, biogas production, waste and drinking water treatment.

In the last decade alone there have been numerous review articles published that deal with cavitation and its ability to destroy microorganisms for purposes of water disinfection [12,18,21–25], food preservation [4,26–28], extraction of bioactive materials [8,10] and its improved efficiency in combination with other methods [29,30].

Most of these review articles focused on acoustic cavitation (AC), which was the first of the cavitation types to be exploited. Also, the focus is usually on either a single microorganism, or a very rudimentary comparison of various cavitation types. Such a limited approach leads to very specific conclusions, which cannot be applied to other cases and are not generally applicable. Unfortunately, they are frequently taken as such, what leads to many dead allies in the progression of this scientific and engineering field.

To maintain the focus, we limited this review to discussion of the mechanical and chemical effects of cavitation on four types of microorganisms (i.e. bacteria, yeast, algae) and viruses in planktonic form. Even though viruses are not living organisms we will name them as microorganisms in this review paper for simplicity. We considered both AC and hydrodynamic cavitation (HC), as many claim that the latter is more efficient. Additionally, we limited the review to the publications from the last decade.

The main goal of this paper is to review and summarize, which mechanisms are possibly responsible for microorganisms' destruction by cavitation. We considered papers that are explaining the mechanisms behind the effects of cavitation exclusively in distilled/deionized water, tap water, surface water, seawater, artificial sea water, salt solutions, growth medium and different buffer solutions. How AC may promote microorganism's growth (as discussed by Huang et al. [31]) is not in the scope of this review.

One problem, which we noticed during the past years, and which persists in many studies on cavitation exploitation is that the understanding of cavitation is taken very lightly. Hence, in Section 2 we put effort into explaining the main differences of two cavitation types. Another issue is, that conclusions of a certain study are many times disseminated over different types of contaminants – for example, it is argued that a certain type of cavitation reactor, which is efficient for pharmaceutical's removal, will also efficiently destroy bacteria. Unfortunately, this is not necessarily always the case. Section 3 describes characteristics of different microorganisms with the focus on their outer layer where the first effects of cavitation should occur. The core of this manuscript however revolves around the discussion of possible mechanisms by which cavitation acts upon microorganisms, mechanical or chemical. This is thoroughly discussed in Section 4. Finally, in Section 5, we underline problems that hinder accurate comparison of the results reported in the literature. In the end we propose guidelines which should be followed to ensure reproducibility and clearness of the studies and to avoid oversimplification of the problem and uncritical dissemination of the conclusions over various application areas. And this should be done from the perspective of different fields including microbiology, chemistry, physics and engineering. Consequently, such guidelines will hopefully enable faster progression of knowledge and technology, without too many dead ends.

2. Cavitation

Cavitation describes a formation of small vapour bubbles (cavities) inside an initially homogeneous liquid medium. It is a rapid physical phenomenon triggered by the sudden decrease in pressure [32]. As the pressure recovers the bubble goes through the violent collapse and possible rebounds. By bubble growth an energy from the surrounding liquid is collected and released by bubble collapses, where extreme conditions can be formed locally. Bubble collapse can cause pressure shocks up to several 100 MPa [33] and if the bubble collapses asymmetrically the so-called microjets with high velocities above 100 m/s can form [34]. In addition the so called hot spots with extreme temperatures in order of several 1000 K [35] can form at the centre of the bubble at its collapse, which can cause the formation of highly reactive radicals [36]. Exact manifestation of cavitation is influenced by liquid properties (temperature, density, viscosity and surface tension) and quality (number of solid particles and amount of dissolved gasses, which can both act as a nuclei).

In general, two types of cavitation are recognized, hydrodynamic and acoustic cavitation. The difference is in the mechanism, which causes the local pressure to drop, while the principles which govern the hydrodynamic bubble and the acoustic bubble are basically the same. Even though there are numerous different expressions for AC frequently used in the literature like ultrasonic cavitation, ultrasonic irradiation, sonication or even, simply, ultrasound (US) they describe the same thing.

2.1. Acoustic cavitation

In the case of AC, the necessary low pressures to break down the liquid and generate cavitation are achieved by the propagation of

acoustic waves. This requires high acoustic frequencies – generally 20 kHz and above.

AC has been utilised for over 40 years [37]. Until the present day, almost every chemical research laboratory is equipped with some type of AC generator. In this chapter, we discuss different AC setups and operating conditions under which the experiments on treating microorganisms are performed. Firstly, we can differentiate the design of AC devices. Ultrasonic waves are usually generated by the piezoelectric transducer, which transforms high-frequency electrical energy into mechanical vibration. The vibrating part can be:

1. A radial probe (also called a sonotrode or horn probe). Sonotrodes are the most common US devices. To operate, the probe of the sonotrode is submerged into the treated liquid. Typical examples of sonotrodes are presented in Al-Juboori et al., Hunter et al. and Liu et al. [38–40]. The probes can be found in various diameters – papers reviewed here use probes with the diameter ranging from 2 mm to 12.7 mm. Majority of the cavitation activity occurs directly below the tip of the radial probe. Since all acoustic energy is transmitted through a small area (the tip of the probe) we can say that sonotrodes generate high-intensity cavitation.
2. On the other hand, piezoelectric transducers can be mounted on the sides of a container. The so called “ultrasonic baths” (as seen in papers by Monsen et al. and Šarc et al. [41,42]) are also frequently used among researchers. The treated liquid is poured into the bath, where it is exposed to cavitation activity. Cavitation structures occur throughout the volume of the bath, in the anti-nodes of the acoustic waves. This type of cavitation is characterized as low-intensity cavitation because the area through which ultrasound is emitted is large. When using US baths, sometimes the bubbles do not actually collapse (especially if high frequencies are used). Instead, the bubbles oscillate for many acoustic cycles. Extreme conditions associated with the bubble collapse do not occur in this case. Instead, oscillating bubbles produce micro-scale eddies, which induce shear stress on nearby objects. Such cavitation is referred to as “stable cavitation”, whereas cavitation that produces the bubble collapses is also named “transient cavitation”.
3. In addition to sonotrodes and ultrasonic baths other unconventional ultrasonic devices exist. These include hollow radial horns [43], Barrel horn [44], vibrating plates [2,45], or devices producing focused ultrasound [46].

The frequencies at which ultrasonic devices emit the acoustic waves also differ. Most studies are using the piezoelectric transducers with excitation frequency 20 kHz [47,48]. However, researchers use also other frequencies, up to the 3.2 MHz [49], which is the highest used frequency reported in this review paper. In this paper, we refer to frequencies up to 100 kHz as “low frequency US” and to frequencies above 100 kHz as “high frequency US”. By using different transducers in the same device, one can achieve simultaneous excitation with two frequencies [16], where combinations of 17 kHz + 33 kHz and 70 kHz + 100 kHz have been used.

Another important parameter in AC research is its “intensity”. There is, however, no exact definition of cavitation-intensity and therefore different approaches how to describe it exist. Usually, researchers correlate the intensity with the power input to the treated liquid. Therefore, one option is to report the rated electrical power of the specific AC device [44,50,51]. Since not all the electrical energy is converted and emitted as acoustic waves, the second group of researchers tries to evaluate the actual acoustic power. Most commonly acoustic power for sonotrodes and ultrasonic baths is evaluated calorimetrically [22,52]. This method assumes, that all acoustic energy input is eventually dissipated as heat. But even if acoustic power is measured, there are four ways how this is reported in the literature. Acoustic power is either expressed as power (in W), as power over volume (in W/mL or W/L), as power over ultrasound emitting area (W/cm^2), or even as total energy emitted per unit of volume (J/mL). When discussing cavitation intensity, it is also important to note how the sample is exposed to the ultrasonic irradiation. Exposure can be either “direct” – the ultrasonic probe is directly submerged into the sample, or in the case of ultrasonic bath the sample is directly poured into the bath [53,54], or “indirect” – where the sample is contained inside a small beaker, vial or some other container. This container with the sample is then placed into a bigger vessel, usually filled with water. Hence, the surrounding water is in direct contact with the surface emitting acoustic waves and thus the sample is indirectly exposed [41,55,56]. Also, in this case the acoustic power is evaluated calorimetrically [55,56]. In the Supplementary material (Table S1), we recalculated all the reviewed data into W/mL.

Furthermore, US devices can be used in continuous mode, as described in Bastarrachea et al. [57] (emit acoustic waves throughout the treatment) or in so-called “duty cycles” – acoustic waves are emitted for specific time. For example, 3 s excitation which is followed by 7 s off time. This cycle is repeated for the time of treatment, as described in Abeledo-Lameiro and Liu et al. [40,58]. Duty-cycles are employed to control the temperature of the samples – in order to eliminate the temperature’s influence on the results. Majority of the papers are treating sample volumes between 100 and 200 mL, where constant temperatures are maintained using water or ice cooling [59]. If the sample volume is large, no cooling is required (for example [43] where 35 L samples are treated). But often the samples are smaller, down to only 1 mL [46] and therefore researchers operate in duty-cycles, which obviously reduces the energy input.

Lastly, we will differentiate two different experimental set-ups employed in AC research. These are “batch” and “flow-through”. Generally, experiments, where AC is harnessed, are in a batch set-up. The treated sample is contained in a beaker or acoustic bath. Because AC zone is rather small, a proper mixing must be provided in order to assure that the whole sample is equally exposed to cavitation activity. While this design is simple, its usability is limited to relatively small samples (as stated, most of the samples are in the range of 100–200 mL). To increase the volume of the sample, some researchers employ the flow-through set-up. Those set-ups have a specially designed smaller cavitation chamber, through which a liquid is fed from a bigger tank. Such arrangements can contain up to 50 L.

The discussed parameters in this chapter together with the respective references are summarised in the Supplementary material and presented in Fig. 1.

2.2. Hydrodynamic cavitation

In case of HC, acceleration of the liquid flow causes local pressure drop, which can trigger the cavitation formation – if the pressure drops below saturated pressure at liquid temperature. Depending on the flow conditions, the size of the formed cavitation bubbles varies usually between a few nm to a few mm (in very specific cases even cm [60]). Flow conditions and geometry of the submerged body define the cavitation behaviour and its characteristics. When the bubble forms, most of the energy is captured in the liquid surrounding the bubble, depending on its size and surrounding liquid properties. This energy is released, when the bubble goes through the pressure recuperation. At the same bubble size, the pressure gradient determines the collapse intensity. Depending on the pressure gradient, this energy is released on a different timescale. Different cavitation conditions result in different effects and intensities. The cavitation properties can vary by pressure distribution along the submerged body, flow velocities and liquid properties (temperature, density, viscosity and surface tension) and quality (number of solid particles and amount of dissolved gasses, which can both act as a nuclei) [32].

HC can be in general divided into: 1.) attached cavitation, 2.) cloud shedding cavitation and 3.) supercavitation (Fig. 2). In the case of attached cavitation, the large number of vapor bubbles are close together

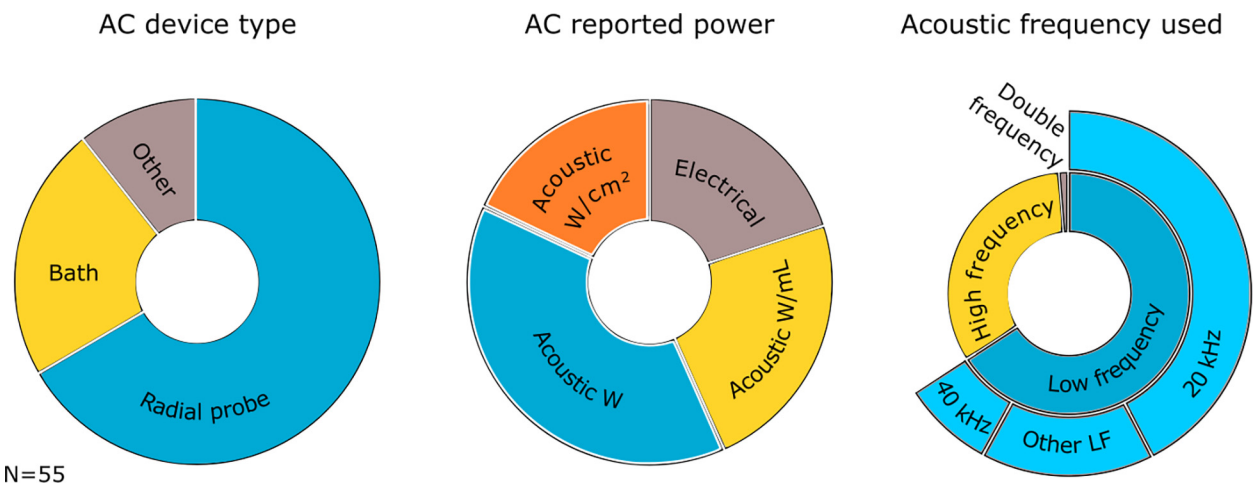

Fig. 1. Papers dealing with AC divided between cavitation device type (left), the reported power (middle) and acoustic frequency the device emits (right); N-number of reviewed papers.

and attached to the leading edge of constriction, forming an attached cloud shape. When flow velocity increases or static pressure additionally drops, the cavitation cloud becomes unstable and starts (partly or completely) to shed from the main cavitation structure (cloud shedding cavitation). If the flow velocity further increases or the pressure drops even lower, the so-called supercavitation forms. It starts when the individual bubbles merge and form a large unified vapor bubble or vapor cloud, which can be stable if the flow conditions do not change once it is formed.

In the reviewed literature three types of cavitation devices were used. They can be divided into: 1.) blow through (BT), 2.) pump + constriction (PC) and 3.) rotor-stator (RS) cavitation devices. Their distribution is graphically presented in Fig. 3, while detailed data is available in Supplementary material (Table S2). Most of the reviewed studies use PC type of devices, where cavitation is most likely already present at the pump impeller, but rare or no remark can be noticed on this issue in the papers. The pump itself is not an issue if it is determined, that it does not cavitate. To exclude the effects of the pump on the results, a BT cavitation device is more suitable. In the case of a BT device, compressed air or a piston is used to push the liquid through the constriction from the first reservoir to the second reservoir. These types of devices are not circular but can be driven as continuously working devices with multiple number of passes with suitable configuration [61,62]. Most configurations with RS devices include pumps for liquid circulation, which can similarly as in the case of PC devices, influence the gathered results. Rare RS designs are capable of operating without additional circulation pump [63].

One of the advantages of HC is its scalability and its potential to be used on the industrial scale. Nonetheless, one must be aware that scaling effects might be difficult to predict [64]. The reviewed papers were divided by sample volume into three sections: 1.) laboratory, 2.) pilot and 3.) industrial scale cavitation devices (Fig. 3 and Supplementary material Table S2).

3. Microorganisms

Microorganisms are microscopic organisms that can exist unicellularly or in a colony of cells. There are prokaryote and eukaryote cell types based on the internal cell structure and other features. Usually prokaryotes are smaller sized than eukaryotes. Prokaryotes have simpler internal cellular organization without enclosed internal membrane structures. Cytoplasm is separated from the surrounding with phospholipid bilayer and extracellular matrix. On the other side, eukaryotes have a more complex structure with additional membrane structures within the cells – called organelles (nucleus, mitochondria, endoplasmatic reticulum, Golgi apparatus, chloroplasts, etc.). Viruses are neither prokaryotes nor eukaryotes since they are not living organisms as they lack many of the attributes of living cells – the most important: they're not a dynamic open system. They also don't have metabolic abilities and replication of their own – they need a host cell [65].

Cell envelope is a sophisticated multi-layered structure and is a

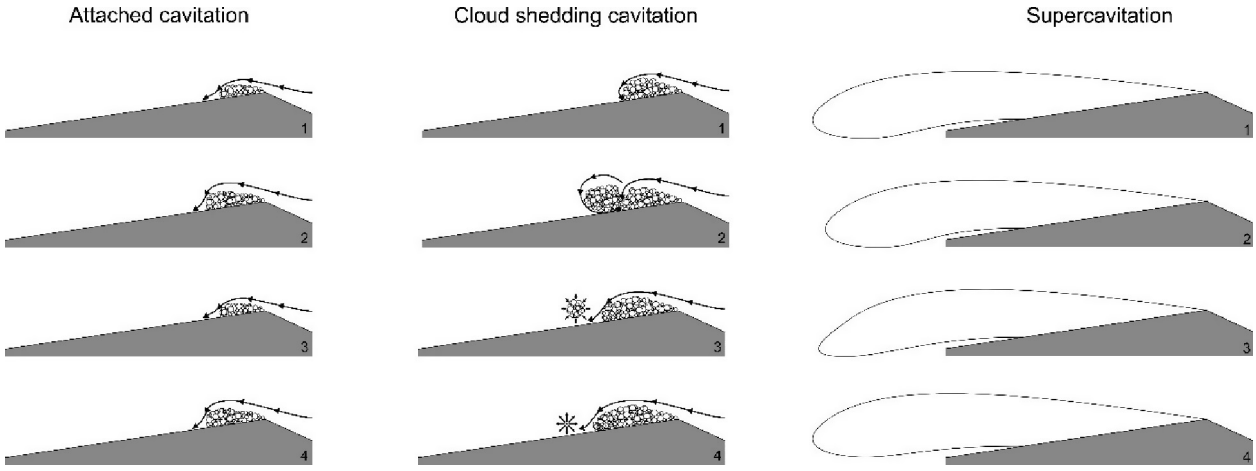

Fig. 2. Types of hydrodynamic cavitation (the flow is from the right to the left).

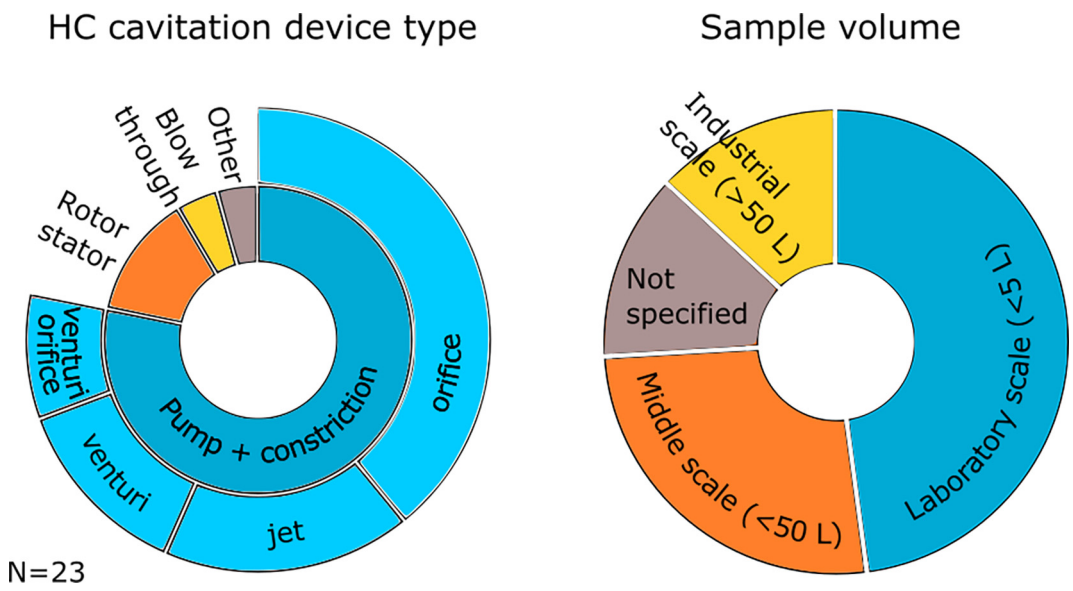

Fig. 3. Papers dealing with HC divided between cavitation device type (left) and sample volume (right); N-number of reviewed papers.

boundary layer between a medium and a cellular cytoplasm [66]. The closest boundary layer to cytoplasm is cytoplasmic membrane. It is a few nm thick and mostly consists of phospholipid bilayer with embedded proteins. Membrane fluidity and heterogeneity are determined with the type of phospholipids, amount of cholesterol and the embedded proteins. Cell membrane can be described as a heterogenic fluid mosaic [67]. Outermost boundary of the cell envelope is an additional layer of matrix called cell wall and gives protection, rigidity and shape to the cell. Cell wall composition varies between microorganisms [65]. Once the membrane is damaged irreversibly, microbial cells will be dead immediately [68]. Therefore, it is important to understand the basics of the cell envelope composition and in this chapter, we describe in detail the outer layer structure of bacteria, yeast and fungi, microalgae, and virus structure (Fig. 4.).

Based on their cell wall composition bacteria can be divided into two groups – gram-negative and gram-positive bacteria. Gram-negative bacteria have complex multi-layered structure of the cell wall. They have an outer membrane, which is a second lipid bilayer with polysaccharides – lipopolysaccharides (LPS). LPS molecule consists of: lipid A, core oligosaccharide and O-antigen [65]. Between the inner and outer membrane layer is a periplasm with a thin layer (3–8 nm) of peptidoglycan [69]. Peptidoglycan is a polysaccharide composed of covalent linked N-acetylglucosamine and N-acetylmuramic acid. Few amino acids (L-alanine, D-alanine, D-glutamic acid, lysine or diaminopimelic acid) are also present in peptidoglycan, which provide a cross-linking of the long polysaccharide chain. Additional cross-linking occurs by the direct peptide linkage of the amino group of diaminopimelic acid to carboxyl group of the terminal D-alanine. As cell membrane is relatively impermeable to small molecules, integrated transmembrane proteins called porins function as channels for transportation of hydrophilic low molecular weight substances [65].

On the other hand, cell wall of gram-positive bacteria mostly consists of a thick single layer of peptidoglycan and is primarily responsible for the strength of the wall [65]. Peptidoglycan layer is 20–80 nm thick [66]. Besides standard peptidoglycan cross-linking, additional cross-linking occurs mainly by peptide interbridges. Many gram-positive bacteria have teichoic and lipoteichoic acid which are embedded in cell wall and cell membrane. Teichoic acid is partially responsible for the negative charge of the cell. Some bacteria can also form endospores, dormant bacterial structures. Their function is a guarantee of the survival of bacteria in harsh conditions (extreme temperatures, low water activity and nutrient depletion). The outer protective layer of endospores consists of exosporium (thin protein covering), spore coats (proteins), cortex (loosely cross-linked peptidoglycan), core wall and cytoplasmic membrane [65]. There are a few irregular bacterial cell wall structures and one of them is present in *Mycobacterium*. It stains gram-positive, but its cell wall shares notable features of gram-negative bacteria as it has a pseudo outer membrane. Mycobacterial cell wall core structure encompasses of mycolyl-arabino-galactan-peptidoglycan [70]. Its cell wall is extremely resistant to drugs (small hydrophilic agents) due to low permeability of the cell wall, has a low number of porin molecules and is extremely rich in lipids [71].

Yeast and fungal cell wall share a similar structure. It has two layers: outer layer consists mostly of mannoproteins, while inner layer is microfibrillar and consists mostly of glucans [72]. These compounds are linked with β-1,3 and β-1,6 bonds. β-1,3 glucan chains are coiled spring-like microfibrillar structures that confer elasticity and tensile strength to the cell wall [73]. β-1,6 glucans are amorphous in structure and act as a flexible glue by cross-linking β-1,3 glucan and chitin to the cell wall mannoproteins [74]. The mechanical strength of the wall is mainly due to the inner layer (glucan and chitin) and represents about 50–60% of the cell wall's dry weight [75].

In the case of cyanobacteria, the cell wall is analogous to gram-negative bacteria. An inner murein or peptidoglycan layer supports and strengthens the wall, while the outer lipoprotein layer controls the transport of molecules. The outer gelatinous sheath is providing protection against exposure to high levels of sunlight [76]. Some cyanobacteria form specialized cells for nitrogen assimilation, called heterocyst. Heterocyst are surrounded with thick cell wall containing large amounts of glycolipids, which slows down diffusion of O_2 into heterocyst to maintain anoxic conditions for nitrogen assimilation [65].

In the case of green algae most of their outer wall has a membrane-like trilaminar structure, which exhibits two electron dense sublayers and one sublayer with low electron density between two electron dense layers. These walls have generally highly aliphatic structure. Most of the cell walls contain biopolymers called "algaenan" – an insoluble biopolymer, which is resistant to drastic non-oxidative chemical treatments [77]. Because algae are a large and diverse group, there is also great diversity in chemical compositions of the cell walls [78].

Diatoms on the other hand, have extremely crush-resistant silica cell wall (frustule). Frustules are of different shapes and typically show morphological symmetry [65]. The silica is a polymerised silicic acid and is amorphous with no crystalline structure [79]. The frustule of diatoms can withstand extreme mechanical forces [80].

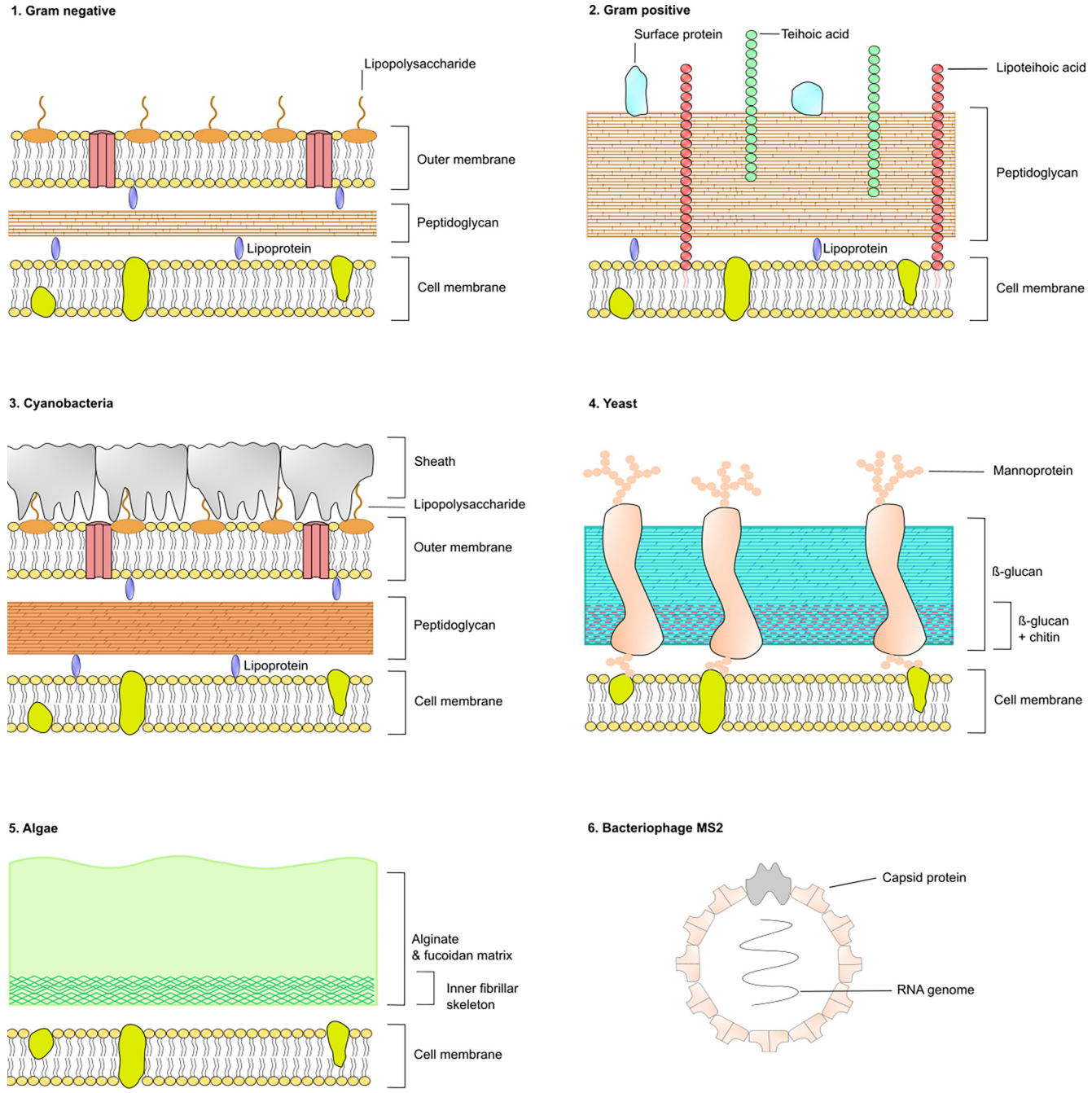

Fig. 4. Cell wall structure of different types of microorganisms and virus structure of bacteriophage MS2. Drawings were adapted from literature [82–85].

Most viruses are smaller than prokaryotic cells, ranging from 20 to 300 nm. Extracellular form poses genome information, surrounded with proteins or other macromolecules, which together form a virus particle. Capsid proteins are monomers or multimers of capsid protein subunits. Viral structure is diverse – usually it is constructed in a highly symmetric way, most common is icosahedral and helical symmetry. Some viruses have additional membrane around capsid – an envelope. Envelope membrane is derived from host cell. Some bacterial viruses possess even more complex structure with icosahedral head and helical tails [65]. High degree of structural diversity of viruses is reflected in a different resistance to physico-chemical treatments. Wide range of pressure resistances is found in-between viruses [81].

4. Cavitation and its possible effects on different microorganisms

Mechanisms responsible for microorganism's inactivation by AC and HC, can be divided into mechanical, thermal and chemical effects [12,20,21,86–88].

4.1. Effects of cavitation

4.1.1. Mechanical and thermal effects

When bubbles in the liquid form and then collapse, extreme conditions can occur that drive the reported mechanical effects such as shock waves, liquid microjets and high shear forces (turbulence and eddies) [59,89–92].

All these mechanical effects are reported to physically damage, weaken or tear the outer layer of different organisms

[12,20,28,91,93,94].

In addition to the above-mentioned effects, in AC another distinctive mechanism – microstreaming, occurs [28,92,95]. By providing high localized shear forces, microstreams can cause serious damage to microorganisms [95] and together with shockwaves generated by bubble collapses facilitate mass transfer processes [92].

Moreover, bubble collapses can cause hotspots – small areas with extremely high temperatures, which can locally damage microorganisms [12,20,92]. Besides this, extreme temperatures can also affect the integrity of the outer layer of microorganisms and make them more susceptible to further damage with reactive species [96].

4.1.2. Chemical effects

Implosion of bubbles and consequently formation of local hot spots is responsible for homolytic cleavage of H_2O molecules and formation of free radicals ($^{\bullet}OH$ and $^{\bullet}H$) [12,91,92]. Being one of the strongest oxidants, $^{\bullet}OH$ readily oxidize any species they encounter or react between themselves forming H_2O_2 [54,91,93,97]. Many other species can form ($^{\bullet}O_2H$, $^{\bullet}N$, $^{\bullet}O$, $^{1}O_2$) when different gases air/oxygen are dissolved in water [92,98].

In the case of acoustic cavitation, the number of radicals that reaches the liquid bulk phase depends on the frequencies employed. It has been established that with low ultrasonic frequencies (100 kHz and below) formed bubbles are bigger and their collapses more aggressive. This on one hand releases more energy in terms of shock waves but on the other hand the number of collapses per unit of time is smaller, which hinders diffusion of $^{\bullet}OH$ into the bulk phase [22,90,92,99,100]. Situation is the opposite for the higher frequencies (above 100 kHz). In this case smaller bubbles are formed, which release less energy. However, the number of collapses is higher, and this facilitates the diffusion of $^{\bullet}OH$ [54,90,92,99].

Similarly, smaller bubbles and more collapses are favourable for the diffusion of $^{\bullet}OH$ radicals into the bulk liquid in the case of HC [90]. The efficiency of HC on cell disruption correlates with bubble pressure collapse which depends on cavitation number, presence of dissolved gases, viscosity of the liquid medium, vapour pressure and above all the design of the HC device [10,11,101,102].

4.1.3. Oxidation of microorganism's constituents

Organisms can defend themselves against oxidative stress but when levels of reactive oxygen species (ROS) exceed their antioxidant capabilities oxidative stress ensues [103,104]. OH radicals and H_2O_2 both act as strong biocides [54,91]. H_2O_2 has been shown to be effective against bacteria, yeast, microalgae and viruses [105–108] but its effectiveness depends on the concentration applied [105,106,108,109]. H_2O_2 toxicity is thought to be a consequence of the production of oxidative species like OH radicals intracellularly, but the exact mechanisms of how these reactive species are formed are not yet fully elucidated [108].

Important constituents of microorganisms, that can be affected by oxidation with different reactive species, are polysaccharides, proteins, lipids and DNA [106,107]. As short-lived and electrophilic species, $^{\bullet}OH$ radicals can attack and oxidise double bonds and sulfhydryl groups of these constituents and *via* chain reactions produce oxidative stress that ends with irreversible consequences for the microorganisms [108–111].

4.1.3.1. Oxidation of proteins. Oxidation of proteins with reactive species can occur in and on the surface of the outer protective layer (protein capsid) as was demonstrated for environmentally important viruses – leviviruses, adenoviruses, caliciviruses and enteroviruses [112,113]. Depending on the oxidant in question protein backbone as well as side chains can be affected with $^{\bullet}OH$ being the least selective [113]. These oxidation reactions affect virus infectivity and replication by hindering normal functioning of viral constituents [114] or by altering the capsid structure and providing access to interior constituents [96].

Oxidation of proteins can also take place intracellularly when radicals are generated inside the cells from H_2O_2 [108]. Once formed, $^{\bullet}OH$ attack electron rich sites like double bonds of the amino acid side chains and backbones [106,110] and can oxidize amino acids such as tyrosine, phenylalanine, tryptophan, histidine, methionine and cysteine. Consequently, the specific function of the corresponding protein is inhibited [108].

4.1.3.2. Oxidation of lipids. When $^{\bullet}OH$ attack polyunsaturated fatty acids of lipids and cause chain reactions that lead to generation of many other ROS it is referred to as lipid peroxidation [104,109]. This has been reported in numerous studies dealing with effects of free radicals and H_2O_2 on bacteria and yeast [104,105,108,109,115]. Once the reaction is started, the bacteria cell membrane integrity is affected. Changes in membrane fluidity, permeability and deterioration of membrane's internal organisation in the end lead to free radicals reaching the interior of the cell and causing additional damage to intracellular components [108,115].

4.1.3.3. Oxidation of polysaccharides. One of the distinctive features of gram-negative bacteria is the outer polysaccharide layer [65]. It is reported in the literature that non-radical (H_2O_2) and radical ($^{\bullet}OH$, $^{\bullet}OOR$, $^{\bullet}OR$ and $^{\bullet}ON$) ROS can attack polysaccharides. By the scission of the glycoside backbone they cause fragmentation of the biopolymer and cause its fragmentation which changes the functionality of these cell constituents [116]. It was also shown that the susceptibility to radical attack depends on the polysaccharide composition as was demonstrated for sulphated polysaccharides [116].

4.1.3.4. Oxidation of nucleic acids. It was shown that also nucleic acids are susceptible to oxidative stress initiated by ROS. Once inside the cells $^{\bullet}OH$ can cause a break of the double helix and/or modify nitrogen bases [111,113].

All relevant available literature data, where unambiguous effectiveness results could be deducted, is summarized in tables below and only the maximum recorded effects are given.

4.2. Effects of cavitation on bacteria

In Tables 1–3 results from reviewed articles are presented. One can observe, that researchers employed different cavitation types, treatment times, medium, initial concentration of bacteria, different bacteria species, and therefore the measured inactivation rates differ vastly. More articles were investigating US than HC, therefore more data is available for US.

Effect of bacterial inactivation with US could depend on different operating parameters of the experimental design. High frequency ultrasound (HFUS) resulted in greater inactivation compared to low frequency ultrasound (LFUS) for *Escherichia coli* and *Streptococcus mutans* [56]. In Table 3 positive correlation between higher frequency and greater inactivation for Staphylococcus epidermidis can be seen. On the other hand, lower inactivation rate authors observed for HFUS in the case of *Mycobacterium* sp. [54] and *E. coli*, *Klebsiella pneumoniae* [55]. Directly comparing these results might be challenging as researchers used different microorganisms, cavitation operating conditions, treatment times and sample volumes. In addition, increasing power intensity also had a positive effect on inactivation rate as most researchers observed in the case of low frequencies [38,88,117,118], high frequencies [56,99] and with both types of frequencies [54]. Holm et al. [119] observed no effect on *Cobetia marina* and *Enterococcus avium*, but they observed positive effect on *E. coli* and *Vibrio cholerae* at low frequency. They do not offer any suggestions regarding conflicting results between different bacteria.

Different cavitation chamber designs can be utilized to achieve HC. Venturi chamber design seems to be more effective for bacterial inactivation compared to the orifice design [90]. However, multi-hole

Table 1
Review of cavitation effects on gram-negative bacteria reported in the literature.

Species	Characteristics (diameter * length, shape)	Medium	Conc. (CFU/ml)	Proposed mechanism	US freq. (kHz)	Effectiveness (%)			Time (min)	Ref.
						LFUS	HFUS	HC		
	Gram-Negative									
Cobetia marina	2.8*1 μm Rod [131]	ASW	2–5 × 10^6	M + C	19	90			3.6	[119]
Enterobacter aerogenes	0.7*3.5 μm rod	SS	/	/	20	4.4[a]			20	[126]
		SS	10^6	M	20	5[a]			40	[88]
		SS	10^8	M + C	850		4.4[a]		20	[99]
Haemophilus influenzae	0.5 μm, round [132]	BS	10^3	/	20	99			10	[41]
Klebsiella pneumonia	0.7*1.6 μm rod [133]	BS	10^5	M	20	90			15	[55]
		BS	10^5	M	580		25		15	[55]
		SS	10^7	M	–			5[a]	60	[93]
Legionella pneumophila	0.5*2 μm rod [134]	BS	1.5–18 × 10^3	M	–			30	60	[42]
		BS	1.5–18 × 10^3	M	33	20			60	[42]
		SS	10^5	M	–			3.6[ac]	60	[60]
Pseudomonas aeruginosa	0.8*3.1 μm rod [135]	BS	1.0°	M	70	2.7[b]			3.6	[118]
		BS	1 × 10^3	/	20	90			15	[41]
		SS	10^8	M	–			3[a]	90	[93]
Pseudomonas putida	0.75*3.2 μm rod	DW	/	M	–			100	5p	[122]
Pseudomonas syringae	0.9*2.6 μm rod [136]	SS	10^7	M	–			6[a]	20	[93]
Salmonella enterica	1*3 μm rod [137]	DW	10^6	/	24	1[a]			0.8	[138]
Vibrio cholerae	0.4*3.1 μm rod [139]	ASW	2–5 × 10^6	M + C	19	90			0.9	[119]
Surface water coliforms	/	SUW	250	/	20	70.8			15	[38]

LFUS: low frequency ultrasound; HFUS: high frequency ultrasound; HC: hydrodynamic cavitation; ASW: artificial seawater; BS: buffer solution; DW: distilled water; SS: salt solution; SUW: surface water; M: mechanical damage; C: chemical damage; a: log reduction; b: rate of nitrocefin hydrolysis (nmol/mg/s); c: supercavitation; p: number of passes; °OD_{700} (a.u.); /: data not available.

orifice improves bacterial inactivation compared to a single-hole orifice [90,101]. In multi-hole orifice design, round shaped holes seem to be the most effective, which is attributed to a higher hole number per cross-section [101]. Loraine et al. [93] observed that different nozzle geometry at the same flow rates and total opening area affect the inactivation rate. Slit venturi design proved to be the most energy efficient design for bacterial inactivation, when compared to multi-hole orifice and cylindrical venturi [120]. Increasing discharge pressure has greater positive effect on inactivation in multi-hole orifice, whereas in venturi it has a negative effect, expressed as lower inactivation rate [90]. Lower cavitation number relates to higher protein release or inactivation rate [93,121]. Increasing nozzle velocity [122], flow rate [101] and cavitation intensity [123] results in an increase of inactivation rates. Loraine et al., Badve et al. and Dalfré Filho et al. [93,120,124] investigated the effect of inlet pressure on inactivation rates. They determined that by elevating the inlet pressure inactivation rates increase but only up to a certain point when a plateau is reached. At further elevation of the pressure the inactivation begins to decrease.

Table 2
Review of cavitation effects on gram-negative bacteria *Escherichia coli* reported in the literature.

Species	Characteristics (diameter * length, shape)	Medium	Conc. (CFU/ml)	Proposed mechanism	US freq. (kHz)	Effectiveness (%)			Time (min)	Ref.
						LFUS	HFUS	HC		
	Gram-Negative									
Escherichia coli	0.5*1.5 μm rod	BS	0.5[e]	M + C	–			70[b]	1000p	[121]
		BS		M	20	95			50	[117]
		GM	1.4 × 10^{11}	M	–			100	3p	[122]
		SS	10^3, 10^4, 10^5	M	–			80	120	[90]
		SS	10^4	/	20	3.9[a]			10	[50]
		ASW	2–5 × 10^6	M + C	19	90			1.4	[119]
		GM	4 × 10^6		20	99			3	[39]
		SS	10^8	C	500		1[a]		10	[56]
		BS	10^8	M	20	4[a]			4	[53]
		BS	1 × 10^3	/	20	90			15	[41]
		BS	10^5	M	20	91			15	[55]
		BS	10^5	M	580		5		15	[55]
		BS	/	M	–			93[b]	1000p	[101]
		DW	10^{4f}	M	–			2.2[a]	50	[127]
		SS	10^7	M	–			5[a]	60	[93]
		TW	10^7	M	–			100	30	[124]
		SS	10^6, 10^8	/	20	98.1			20	[125]
		DW	/	C	26	1.7[a]			3	[1]
		DW	10^6	M + C	–			100	8	[86]
		GM	10^8	M	20 + 33	6[ac]			60	[47]
		GM	10^9	M + C	20	89.1			12	[68]
		GM	10^7	M + C	–			100	14	[87]
		SS	10^8	M	–			3.3[a]	150	[60]

LFUS: low frequency ultrasound; HFUS: high frequency ultrasound; HC: hydrodynamic cavitation; ASW: artificial seawater; BS: buffer solution; DW: distilled water; GM: growth medium; SS: salt solution; TW: tap water; M: mechanical damage; C: chemical damage; a: log reduction; b: release of acid phosphatase; c: dual frequency; p: number of passes; /: data not available.

Table 3
Review of cavitation effects on gram-positive bacteria reported in the literature.

Species	Characteristics (diameter * length, shape)	Medium	Conc. (CFU/ml)	Proposed mechanism	US freq. (kHz)	Effectiveness (%)			Time (min)	Ref.
						LFUS	HFUS	HC		
	Gram-Positive									
Bacillus globigii (spores)	0.6 µm [140]	ASW	2–5 × 10^6	M + C	19	90			213	[119]
Bacillus halodurans	0.75*2.5 µm rod	DW	/	M	–			100	6p	[122]
Bacillus subtilis	0.75*2.5 µm rod	GM	1.4 × 10^{11}	M	–			100	4p	[122]
		SS	10^7	M	–			5[a]	120	[93]
		SS/GM	/	/	20	4.5[a]			20	[126]
		SS	10^8	M	850		2.5[a]		20	[99]
		SS	10^5	M	–			3.8[a]	120	[60]
Enterococcus faecalis	0.8*1.1 µm ovoid [141]	BS	103	/	20	75			60	[41]
		DW	10^6	M + C	–			100	10	[86]
Enterococcus avium	1 µm ovoid [142]	ASW	/	M + C	19	90			20.1	[119]
Lactobacillus acidophilus	0.6*3.5 µm rod	SS	10^4	/	20	0.6[a]			10	[50]
Listeria innocua	0.3*1.3 µm rod [143]	GM	10^8		20 + 33	4[ab]			60	[47]
		GM	10^6	M	20	3.2[a]			35	[57]
Mycobacterium species Strain (6PY1)	0.4*5 µm rod	GM	0.5[b]	M	20	93			70	[54]
			0.5[b]	C	612		43		70	[54]
Staphylococcus aureus	1 µm coccus [144]	BS	10^3	/	20	40			60	[41]
		SS	10^6, 10^8	/	20	91.7			20	[125]
		GM	10^9	M + C	20	81.3			12	[68]
Staphylococcus epidermidis	0.8*1.0 µm coccus	BS	10^3	/	20	20			60	[41]
		SS	/	/	20	0.2[a]			20	[126]
		SS	10^8	C	–			4.4[a]	20	[99]
Staphylococcus pseudintermedius	0.6 µm coccus [145]	SS/GM	/	/	20	0.2[a]			20	[126]
Streptococcus mutans	0.3 µm coccus [146]	SS	10^8	C	500		2[a]		22	[56]
Seawater bacteria	/	SW	10^7	M + C	–			44	15	[120]

LFUS: low frequency ultrasound; HFUS: high frequency ultrasound; HC: hydrodynamic cavitation; ASW: artificial seawater; BS: buffer solution; DW: distilled water; GM: growth medium; SS: salt solution; SW: seawater; M: mechanical damage; C: chemical damage; a: log reduction; b: dual frequency; p: number of passes; /: data not available.

Higher initial cell concentrations led to lower inactivation rates in many of the reviewed articles in AC treatment [39,90,122]. However, Li et al. [125] and Al Bsoul [54] reported no difference between initial concentration on inactivation after 20 min and 70 min treatment, respectively. Loraine et al. [93] observed that with higher initial bacterial concentration, only longer treatment time is needed to achieve comparable inactivation rates at investigated concentration. This hypothesis could answer conflicting results mentioned above. The same correlation of initial concentration and inactivation rates can be seen for different types of HC devices: it has a greater effect for orifices, but only a small effect for venturi geometry [90]. Li et al. [125] speculated about the possibility that with higher cell density ultrasonic waves could act on a higher proportion of bacteria. On the other hand, they suggested also restricted effect of cavitation due to greater aggregation of bacteria.

Moreover, effect of bacterial inactivation could depend on the characteristics of used bacteria. Gao et al. [99] suggested that inactivation of *Bacillus subtilis*, *via* the breakage of cell wall, with HFUS is a result of rod-shaped cells. They observed lower inactivation rate in the case of LFUS compared to HFUS. Cameron et al. [50] observed unique destruction of rod-shaped *Lactobacillus acidophilus* where most damaged cells had “sheared off” tip of the cells. Gram-positive bacteria seemed to be more resistant to cavitation in comparison to gram-negative. This is assumed to be due to a thicker, more rigid and robust properties of their cell wall [47,50,68,86,93,122,125,126]. Li et al. [125] concluded that primary target for gram-negative bacteria is the outer membrane, while in the case of gram-positive bacteria the target might be cytoplasmic membrane and internal cell structure. Differences between the same gram staining group could be ascribed to the level of cross linking in the outer layer [93]. On the other hand, Gao et al., Koda et al. and Holm et al. [56,88,119,126] did not observe any differences in the destruction of bacteria based on gram staining, shape or size of bacteria. Therefore, Gao et al. [126] proposed thickness of the capsule (cell wall) as one of the physical characteristics which might be responsible for this observation. Capsule is soft and consequently dampens the shear forces pointed toward the cell membrane. Additionally, they proposed extent of bacterial surface hydrophobicity as a very important parameter for US treatment. Surface of the cavitation bubble is hydrophobic and cavitation bubbles will be attracted to hydrophobic surfaces. Additional protective structures may play a role in bacterial resistance to cavitation treatment as spores of *Bacillus golbigii* are extremely resistant to ultrasound – at least a magnitude higher decimal reduction time was observed [119].

Most of the investigated bacteria were inactivated to more than 70%, but there are also cases where achieved inactivation was not considerable (Tables 1 and 3). For example, Gao et al. [126] observed low inactivation of *Staphylococcus epidermidis* with LFUS (0.2 log after 20 min of treatment). They showed that these bacteria form clusters and observed no effect on structural changes in cells after US treatment. Badve et al. [120] also achieved low inactivation of total bacteria in seawater. In the case of HC, some authors achieved total inactivation of bacteria as a result of treatment. Gram-positive bacteria are probably more resistant to cavitation treatment, as low inactivation can be seen for many species (Table 3). Interestingly, experiments with *Legionella pneumophila* (gram-negative bacteria, Table 1) also show low inactivation in the case of AC or HC, but greater inactivation with supercavitation type of HC [42,60]. They suggest rapid pressure drop as a possible mechanism [60]. Interestingly for *Staphylococcus aureus* contradictory results are reported (Table 3). Monsen et al. [41] showed only 40% inactivation, whereas Li et al. and Liao et al. [68,125] achieved higher inactivation rates (~81.7%) in shorter treatment time. This dissimilarity of results might be due to different US set-ups – ultrasonic bath and sonotrode, respectively.

A lot of authors observed the effect of cavitation using microscopic techniques (Transmission Electron Microscopy – TEM and Scanning Electron Microscopy – SEM). They concluded that cavitation caused membrane ruptures and damage to the cell wall, morphological changes of cells, empty cell envelopes, occurrence of large vacuoles, cell fragments and shrinkage of cells, etc. [50,53,55,56,68,88,99,121,125,126]. On the other hand, Gao et al. and

Koda et al. [56,99] observed that in the case of *Enterobacter aerogenes* and *Streptococcus mutans* most cells had deformed shape (loss of turgor pressure) but without visually ruptured cell wall after HFUS treatment. Only small number of cells were visually damaged even though inactivation rate was high. Similar observations were also made by Li et al. [125] for *E. coli* with LFUS treatment as a consequence of cell wall composition. It was postulated that HFUS has mechanical and chemical effect on bacteria [99]. Interestingly, Cameron et al. [50] observed small vesicles (< 20 nm) inside and outside of cells as a result of US treatment. They proposed that formation of vesicles was probably a result of emulsification of membrane lipids with cavitation air bubbles. HC seems to selectively damage bacterial cells. Balasundaram and Harrison [121] postulated that this selectivity is due to formation of smaller punctures in the "boundary layer". They are formed on the outer cell wall of bacteria due to the microjets and result in the leakage of periplasmic enzymes out of the cell. Additionally, they proposed that with different number of passes, different cellular damage can be achieved. At first, outer cell membrane is damaged due to the mechanical effects of cavitation. Then, mechanical and chemical damage occurs in the inner cytoplasmic membrane. Lastly, with longer exposure of the cells to cavitation, more effects can be observed on the outer cell wall. This is probably due to its greater surface area being exposed to the medium. Runyan et al. [118] made similar hypothesis that US can perturb the cell membrane and improve permeability of large hydrophilic molecules as are antibiotics. Šarc et al. [42] proposed that destruction effect in the case of supercavitation is a combination of rapid pressure change and exposure of all the treated volume to the tension forces. Liao et al. and Li et al. [68,125] observed no sublethal subpopulation of damaged cell and therefore concluded that US treatment might be "all or nothing" phenomenon. Cerecedo et al. [86] reached similar observations for HC treatment. Interestingly, Mezule et al. [123] made different observations of *E. coli*, treated with HC. They observed sublethal population, which was metabolically active, but not able to elongate (viable but not culturable cells – VBNC). Liao et al. [68] utilized intriguing insight into the process by monitoring cell membrane potential. They observed a change of membrane potential during treatment with induced peaks after 5 min for *E. coli* and 3 min for *S. aureus*. Cell membrane potential phenomenon might be related to the change of ion channels. Although most authors concluded mechanical and chemical effects are responsible for bacterial destruction, interestingly Spiteri et al. [1] showed involvement of heat shock response genes in US treatment.

In some of the reviewed articles cell destruction was contributed to chemical effect. In the case of HFUS chemical mechanism of destruction might be predominant [56,99]. Additionally, Al Bsoul et al. [54] investigated also formation of H_2O_2 in distilled water during US treatment. During HFUS and LFUS treatment they detected 148 μM and 15 μM of H_2O_2, respectively. Chemical damage was mentioned also for LFUS as Liao et al. [68] suggested that part of cells was damaged intracellularly – ATP level decrease and DNA damage. Their results showed that some cells had intracellular DNA broken and enzymes inactivated without disruption of the cell wall. As a possible mechanism they proposed injection of ROS with cavitation microjets into the cells without damaging cell envelope. Even more compelling are results from Gao et al. [99] where they observed that mechanism of bacterial inactivation with HFUS depends on bacterial species. They concluded that mechanism of inactivation in the case of *B. subtilis* was still predominantly mechanical, while for *S. epidermis* and *E. aerogenes* it was chemical. Spiteri et al. [1] investigated effects of LFUS on different *E. coli* mutants with different gene deletion affecting specific functional properties. They determined that the strain without o*xyR* gene was more resistant to treatment. Since *oxyR* activated genes have direct or indirect antioxidant functions, these results are somewhat interesting. The authors explained this could be due to instability of H_2O_2 produced during treatment. Besides production of ROS, production of H_2O_2 is also correlated with decrease of medium's pH. This is the consequence of nitrous, nitric acid and carbonic acid formation because of dissolved gases [99]. Gashchin and Viten'ko [127] on the other hand offered the following explanation. Bacterial inactivation could be due to chemical instability in the cell wall and membrane, rapid penetration of chemical disinfectants inside the cell, change of pH to alkaline side and formation of OH radicals due to Fenton reaction. Together with the explanations and observations of the chemical effects of cavitation on bacteria inactivation there is also a lot of data available in the literature that investigates the effects of oxidants like H_2O_2 and $^{\bullet}OH$ alone on bacteria, mostly on *E. coli*. Labas et al., Flores et al. and Watts et al. [108,111,128] determined toxicity of a H_2O_2 solution on *E. coli.* They proposed that it is not H_2O_2 by itself that causes oxidation of cell's constituents, but the reactive species derived from it [108,111]. Being a small molecule H_2O_2 can diffuse through the cell membrane and transform to $^{\bullet}OH$ via Fenton or Haber-Weiss reaction intracellularly [104,109,129,130]. Whether these reactions take place depends on the presence and amount of superoxide ions and Fe^{2+} intracellularly. Flores et al. [111] discussed that the main site of attack of ROS is bacteria outer layer namely the peptidoglycan layer, lipopolysaccharide layer and phospholipid bilayer. They proposed a model describing the mechanism of bacteria's disinfection by H_2O_2. First attack of $^{\bullet}OH$ on the cellular wall is followed by a second attack of $^{\bullet}OH$, which results in complete destruction of the cell's outer layer and formation of a lysate from all cell components. On the other hand, Kobayashi et al. and Rahman et al. [109,115] investigated effects of $^{\bullet}OH$ formed via different treatment processes. Rahman et al. [115] proposed that $^{\bullet}OH$ formed during sonocatalytic TiO_2 process were responsible for observed lipid peroxidation of *E. coli* cell's membrane, while Kobayashi et al. [109] observed toxic effects of different ROS formed from H_2O_2 under AC on *E. faecalis*.

4.3. Effects of cavitation on yeast and fungi

As can be seen from Table 4 quite a lot of data is available for *Saccharomyces cerevisiae*. Authors either measured the release of intracellular or cell wall constituents but only a small amount of data is available where cell reduction was determined. For *S. cerevisiae* only articles using LFUS were found, whereas for *Aeureobasidium pullulans* also HFUS was tested.

A lot of authors concluded that higher US intensities lead to increased release of proteins [40,147–149], polysaccharides [149] and log reductions [88]. Wu et al. [150] additionally observed that also which constituent is released first, depends on intensity. They showed that at lower intensities polysaccharides were released faster than proteins and at higher intensities it was the other way around. Additionally, Gao et al. [99,126] showed that in the case of *A. pullulans* LFUS [126] is more effective than HFUS [99]. Liu et al. and Iida et al. [40,148] for example showed that horn-type sonotrode is more effective than ultrasonic bath. Bystryak et al. [44] additionally showed that higher sonotrode amplitudes provided higher shear forces, which leads to faster and higher release of cell constituents and represents an important parameter in extraction of membrane-bound proteins.

For HC only two articles, both investigating *S. cerevisiae*, were found. Both authors investigated the effect of cavitation number. Balasundaram and Harrison [89] showed that cavitation number affected the release of soluble proteins and extracytoplasmic but not cytoplasmic enzymes. They determined that lower cavitation number is preferable as it results in more intensive cavitation conditions. Albanese et al. [151] on the other hand determined a two-peak model, where yeast cell damage was observed at low cavitation number and then again at very high number. They also concluded that venturi constriction was more effective than orifice plate.

Several authors investigated the effect of cell's initial concentration on cavitation effectiveness. Iida et al. and Zhang et al. [148,149] showed that with increasing the initial cell concentration the amount of protein released decreased, since the number of cavitation bubbles

Table 4
Review of cavitation effects on fungi and yeast reported in the literature.

Species	Characteristics (diameter, shape)	Medium	Conc. (CFU/mL, g cells/mL*, cells/ml●, w/v (%)°)	Proposed mechanism	US freq. (kHz)	Effectiveness (%)			Time (min)	Ref.
						LFUS	HFUS	HC		
Aureobasidium pullulans (F)	2–13 μm elongated thin walled	SS	10^7	M	20	3[a]			60	[88]
		SS	4.2×10^7 3.1×10^5	M	850		$< 1^a < 2^a$		60	[99]
Saccharomyces cerevisiae (Y)	5–10 μm oval	BS	0.5°	M + C	–			29[b] 39[i] 27[j]	1000p	[89]
		BS	0.09*	/	/	85[b]			15	[147]
		SS	10^4	M	20	3.6[a]			10	[50]
		DW	0.01*	M	20	75[c]			5	[148]
		SS	10^7 ●	M	20	42[d]			5	[24]
		DW	/	/	20	0.6[e]			21	[40]
		DW	0.1*	/	20	10[f] 20[g]			5	[149]
		GM	20°	/	20	18[e]			65	[44]
		DW	0.1*	M	20	92[b] 84[h]			30	[150]

LFUS: low frequency ultrasound; HFUS: high frequency ultrasound ; HC: hydrodynamic cavitation; F: fungi; Y: yeast; BS: buffer solution; DW: distilled water; GM: growth medium; SS: salt solution; M: mechanical damage; C: chemical damage; a: log reduction; b: protein release (%); c: protein release (mg/g); d: tot cell population decline; e: protein release (mg/ml); f: polysaccharide release (dry weight basis: %): g: protein release (dry weight: %); h: polysaccharide release (%); i: extracytoplazmic enzyme release (%); j: cytoplasmic enzyme release (%); p: number of passes; /: data not available.

available for each cell decreased. Similarly, Gao et al. [99,126] showed that the *A. pullulans* inactivation was higher at lower initial concentrations in the case of LFUS and HFUS. They postulated that either viscosity or cell aggregation could be responsible. Higher viscosity lowers the cavitation initiation and higher cell number leads to more cell aggregation and thus bigger resistance to acoustic cavitation. On the other hand, Wu et al. [150] showed that the total amount of released constituents increases with increased concentration but is not correlated to volume. Apar and Özbek [147] found no correlation between effectiveness and cell concentration, while Balasundaram and Harrison [89] showed that an optimal cell concentration exists where the number of interactions between cavities and cells is the highest and leads to the highest release of constituents.

There is no correlation in the literature data proposed between cell shape, size and cavitation effectiveness but for example Iida et al. [148] discussed that the strength of cell wall could play an important role in cell's susceptibility to destruction *via* forces released during cavitation.

There is not a unique mechanism for cell destruction proposed. Balasundaram and Harrison [89] determined that cavitation only affected the release of cell wall bound and periplasmic (i.e. extracytoplasmic) enzymes. They postulated that the cell wall disruption is in this case the consequence of mechanical effects of cavitation like microjets and shock waves, which caused radial wall motion. Due to these effects, smaller holes in the cell wall developed which led to release of only periplasmic constituents but not bigger intracellular macromolecules. The same cell wall punctures were observed by Cameron et al. [50] who also proposed the same mechanical effects to be the culprit. Similarly, Iida et al. [148] discussed how mechanical effects of cavitation and the strength of microbial cells could be correlated. Since yeast cell are rigid microstreaming is not enough to disrupt them and the rupture will occur only when yeast cells are in proximity of cavitation bubbles. Zhang et al. and Wu et al. [149,150] proposed that the cell wall is initially weakened and broken down followed by the breakage of cell membrane.

Some authors also observed changes in microalgae using SEM and TEM. Balasundaram and Harrison [89] observed only localised cell wall damage and not complete cell disruption. Cameron et al. [50] on the other hand, observed cell fragmentation together with internal damages, uneven cell walls, many cells devoid of content and damages to cell microstructures. Similarly, Wordon et al. [24] observed damages on the outer cell layer, fractioning and extrusion of intracellular constituents. Also, Gao et al. [88] observed both broken and intact cells and Gao et al. [99] observed some cell envelopes that could be the result of the damage to the yeast cells resulting in the leaking of their inner contents.

As can be seen from Table 4 not many authors investigated the correlation between oxidants formed during cavitation and destruction of yeast cells. Only Balasundaram and Harrison [89] postulated that radicals play an important role. They speculated that after longer treatment times imploding cavities weaken cell walls to a degree where free radicals can break disulphide bonds and result in release of cell wall bound enzyme invertase. There is however data available in the literature that investigates the effect of different oxidant on yeast cells. Steels et al. [105] demonstrated that H_2O_2 negatively affects survival of *S. cerevisiae* and that outer layer lipid composition plays an important role in cells susceptibility to oxidation. Moradas-Ferreira et al. [103] summarized that H_2O_2 triggers lipid peroxidation of yeast cell membranes. Brennan et al. [152] observed that H_2O_2 causes intrachromosomal and interchromosomal recombination leading to mutagenesis, where $^{\cdot}OH$ is supposed to form in vivo from H_2O_2 via the metal-catalysed Haber-Weiss reaction. Wang et al. [153] postulated that formation of reactive species like $^{\cdot}OH$ during plasma treatment are responsible for triggering chain oxidative reactions in cell constituents like cell wall, cell membrane and DNA and eventually inactivation of yeast cells.

4.4. Effects of cavitation on microalgae

Microalgae are a group of photosynthetic microorganisms consisting of cyanobacteriae, diatoms and unicellular algae. As can be seen in Tables 5 and 6 microalgae are in general susceptible to inactivation regardless of cavitation type and HFUS appears more efficient than LFUS for many species. There are however some contradictory results in the literature in the case of *Microcystis* sp., *Nannochloropsis* sp. and *Scendesmus* sp. even though the same medium and mostly the same microorganism's concentration range was used. Using LFUS Joyce et al. and Lürling and Tolman [3,154] detected no significant damage to *Microcystis aeruginosa*, whereas Zhang et al. [155] observed substantial growth inhibition only after 15 days. In the case of *Nannochloropsis* sp. Kurokawa et al. [59] observed very high cell reduction with HFUS while Wang et al. [49] postulated cell structure changes as higher fluorescence of chlorophyll and lipids was emitted when LFUS was used. Besides low immediate damage Kim et al. [156] also determined only slight growth inhibition after 7 days. *Scenedesmus sp*. proved resistant to destruction with AC [154] while Batista et al. [157] achieved very good results with HC. Wang et al. [49] on one hand determined

Table 5
Review of cavitation effects on cyanobacteria and diatoms reported in the literature.

Species	Characteristics (diameter, shape)	Medium	Conc. (cell/mL wt (%)*)	Proposed mechanism	US freq. (kHz)	Effectiveness (%)			Time (min)	Ref.
						LFUS	HFUS	HC		
Anabaena species (CN)	3.3–9.5 μm oval Prasanna et al. (2006)	GM	/	M	200		92[a]		60	[165]
Chaetoceros species (D)	2.3–2.5 μm	/	1.1×10^8	M	2200	99[b]	99[b]		2	[59]
					20				10	
Microcystis species (CN)	4–5 μm oval to spherical [166]	GM	/	M + C	–			64[c]	8p	[163]
		GM	10^9	M	25	11[b]			5	[155]
		GM	1.5×10^5	M	20	90[b]	90[b]		102	[159]
					1320				20	
		GM	/	M + C	20	Neg	47[b]		20	[3]
					580				30	
		GM	2×10^6	M	20	68[b]			10	[19]
		GM	6×10^6	M + C	1146	39[b]	92[b]		30	[29]
					20					
		GM	1.7×10^6	M + C	–			20[b]	20	[158]
		GM	3×10^6	M + C	–			88[d]	10	[125]
		GM	4.9×10^5	/	–			99[b]	18p	[167]
Thalassiosira pseudonana (D)	5 μm	ASW	0.004*	/	20	85[b]			1.7	[48]

LFUS: low frequency ultrasound; HFUS: high frequency ultrasound; HC: hydrodynamic cavitation; CN: cyanobacteria; D: diatom; ASW: artificial seawater; GM: growth medium; M: mechanical damage; C: chemical damage; a: brightness increase; b: cell reduction; c: cell reduction after 6 days; d: cell reduction after 3 days neg: increased cell concentration; p: number of passes; /: data not available.

higher cell concentration which they ascribed to declumping effect but at the same time proposed cell structure changes due to higher fluorescence of chlorophyll and lipids emitted.

A lot of the authors concluded that the effect of cavitation on microalgae depends on the US frequency [3,49,52,59,158,159] and that higher intensities and longer exposure times correlate to more effective cell destruction [3,19,46,52,154,158–162]. Higher intensities result in more aggressive cavitation which leads to better inactivation but only to a certain point. Excessive intensities can result in formation of too many cavities which than interact between themselves resulting in the cushioning effect [162]. Longer exposure times also showed a positive effect on removal of microcystins [19].

Most authors agree that higher inlet pressure [17,157,163] gives the best results in the case of HC. Higher inlet pressure leads to formation of more bubbles and more aggressive bubble collapses which explains the better efficiency [157,163]. There are however contradictory results about the effect of cavitation number on efficiency. Wu et al. [158] determined higher destruction in the case of increasing cavitation number which they ascribed to higher turbulence. On the other hand, Batista et al. and Xu et al. [157,163] determined better efficiencies with decreasing cavitation number.

Some authors also commented on the correlation between cell concentration and cavitation effectiveness. Xu et al. [163] showed that lower initial concentration yielded better results. Kim et al. [156] observed that in diluted samples the damage to cells is less since interactions between cells and cavitation bubbles are diminished. Halim et al. [161] determined an open-down parabolic relationship between cell destruction and initial concentration. On the other hand, Greenly and Tester [48] showed that concentration does not play an important role but postulated that the volume of the sample may be important for cavitation efficiency. They determined that in the first few seconds the breakage of *Chlamydomonas sp*. cells is the highest.

Table 6
Review of cavitation effects on algae reported in the literature.

Species	Characteristics (diameter, shape)	Medium	Conc. (cell/mL, wt (%)*)	Proposed mechanism	US freq. (kHz)	Effectiveness (%)			Time (min)	Ref.
						LFUS	HFUS	HC		
Chlamydomonas species	3–10 μm spherical thin cell wall	GM	5.5×10^7–10^8	/	1100		85[a] 75[b]		7	[46]
		GM	0.007*	/	20	85[c]			1.7	[48]
		GM	10^7	M	20	85[c]	99[c]		5.2	[52]
					1146				2.5	
Chlorella kessleri	2–3 μm ellipsoidal. [169]	GM	1.5×10^6	/	–			neg	18p	[167]
		GM	2×10^6	/	20	0[c]			20	[19]
Chlorococcum species	10–15 μm ellipsoidal thick cell wall [170]	TP	9.5×10^3 65.5×10^3	/	40	5[c]			25	[164]
Dunaliella salina	1.5–3 μm ovoid irregular cell wall	GM	10^7	M	1146	99[c]	99[c]		1.1	[52]
					20				5.2	
Isochrysis galbana	5 μm lacking cell wall	TP	0.5*	/	20	85[c]			0.4	[48]
Nannochloropsis species	1.3–4 μm spherical robust cell wall	TP	0.5*	/	20	55[c]			2	[48]
		/	1.1×10^{10}	M	20	19[c]	90[c]		10	[59]
					4300					
		GM	1.9×10^8	/	–			13[d]	4p	[156]
Scenedesmus species	6–8 μm bean shaped [171]	GM	5.2×10^4	M + C	–			85[c]	60	[157]

LFUS: low frequency ultrasound; HFUS: high frequency ultrasound; HC: hydrodynamic cavitation; GM: growth medium; TP: tap water; M: mechanical damage; C: chemical damage; a: chlorophyll release; b: protein release; c: cell reduction; d: dry cell weight reduction; neg: increased cell concentration; p: number of passes; /: data not available.

From the data summarized in Tables 5 and 6 there doesn't seem to be any obvious correlation between the size and shape of the cell and its destruction. However, Wang et al. [49] proposed that cell size, shape or structure could all play an important role in microorganism's destruction when they determined different susceptibility of *Nannochloropsis sp*. and *Scenedesmus* sp. There might however be some correlation between cell wall structure and cavitation efficiency. For example, *Chlamydomonas* sp. and *Dunaliella salina* which have a thin and irregular cell wall, respectively, were both destructed to a high degree with all AC frequencies investigated. Greenly and Tester [48] observed that *Nannochloropsis oculate*, which has a robust cell wall, is the most resilient of the species tested. The only microalgae that did not seem to be affected by cavitation were *Chlorella kessleri* and *Chlorococcum* sp. Rajasekhar et al. [19] postulated that this is due to the lack of gas vacuoles, while Halim et al. [164] postulated that the thickness of the cell wall was the culprit. Kim et al. [156] proposed that lower reductions of *Nannochloropsis salinas* were the result of the lack of gas vacuoles and cell structure which is mostly composed of algaenan and cellulose, that give it a more robust structure. Whether cell wall composition is the determining factor in microorganism's susceptibility to destruction with cavitation should be investigated more thoroughly.

Most authors still report that cyanobacteria destruction is in one way or another correlated to the rupture of gas vacuoles which is also in accordance with prior literature. With gas vacuoles ruptured the cyanobacteria lose their ability to float and start sinking to the bottom. Not getting enough light eventually leads to their demise. Gas vacuoles can presumably rupture when the resonance size of cavitation bubble and gas vacuoles are of the same size range [159]. Zhang et al. [159] calculated that at HFUS the sizes are in the same range which could be the reason for their better efficiencies observed in the case of *Microcystis* sp. Jachlewski et al. [43] for example observed that cells without gas vacuoles were also susceptible to HFUS and Jančula et al. [167] achieved the destruction of gas vacuoles without damages to the membranes with HC (no release of toxins). Rajasekhar et al. [19] observed faster effects of LFUS towards *Anabaena* sp. as compared to *Microcystis* sp. under the same treatment conditions, which they ascribed to the former having weaker vacuoles. They also observed no effects on *C. kessleri* which lacked gas vacuoles. Rodriguez-Molares [168] observed immediate destruction of gas vacuoles with LFUS but also their recovery after 24 h. Their recovery however didn't influence the cyanobacteria sedimentation which was still progressing but at a slower pace. To conclude, Lürling and Tolman [154] summarized that the accuracy of calculations of the resonance frequency in the literature is doubtful, which is why more effort should be put into investigation of this.

Kotopoulis et al. [165] postulated a similar mechanism in the case of *Anabaena sp*. where the rupture of heterocyst's membrane is the consequence of US waves coming in resonance with it. Yamamoto et al. [52] proposed the same mechanism in the case of algae *Chlamydomonas concordia* cells, where the radii of the bubble generated by HFUS is supposed to be of the same size range as the algae cell, meaning it causes mechanical resonance of the cells and leads to their destruction. Kurokawa et al. [59] calculated resonance frequencies for *Chaetoceros* sp. and *Nannochloropsis* sp. and determined that they correlate well with the most effective frequency tested.

There are some other mechanisms proposed in the literature. One possibility for microalgae destruction could be the damage of the photosynthetic system (i.e. psycocyanins, chlorophyll) [17,29,43,155,163], which if compromised hinders cyanobacterial growth. Li et al. [17] for example determined a decrease of zeta potential for *Microcystis aeruginosa* after cavitation and proposed that this could lead to cell's instability and enhanced settleability. Jančula et al. [167] on the other hand observed no changes in metabolic activities after HC. Lee et al. [11] investigated *Tetraselmis suecica* and determined that HC causes periplasmic cell disruption with cell bodies intact (i.e. confined to cell wall and membrane) whereas AC causes cytoplasmic disruption with cells split open.

A lot of authors also determined changes in microalgae using SEM and TEM. Rajasekhar et al. and Lürling and Tolman [19,154] observed disrupted filaments which can inhibit the growth of *Anabaena* sp. and *Cylindrospermopsis raciborskii*. Halim et al. [164] observed no changes to *Chloroccocum* sp. under the microscope but they observed complete destruction of *T. suesica*. Xu et al. [163] observed several crucial changes in *M. aeruginosa*. There were changes in the internal layers of the cells, separation of cytoplasm from the cell, condensation and disrupted arrangement of thylakoid membrane in the centre of the cell and destruction of gas vesicles. Jachlewski et al. [43] detected dark membrane-like structures instead of gas vacuoles in natural samples of *M. aeruginosa*. In the laboratory culture that did not contain gas vacuoles they observed severe damage to the outer cell membrane. Li et al. [17] observed smooth cell surface, stripped of extracellular organic matter (EOM), cell rupture after 30 min and cell disintegration after 60 min exposure time. They also observed clear depressions on the cells which pointed to destruction of gas vacuoles. Adam et al. [6] observed changes on the surface of the *N. oculata* cells after LFUS treatment. They also observed disruption of the cell wall which they ascribed to physical effect of cavitation. Batista et al. [157] observed irreversible morphological damages caused to *Scenedesmus* sp. together with cell wall lesions, cavity formation and loss of flotation parts. On the other hand, Bigelow et al. [46] did not use the microscope but only postulated that complete cell fragmentation of *Chlamydomonas sp*. must take place because of the release of proteins and chlorophyll normally located inside the cells.

There are also inconsistencies in the literature whether radicals give any contribution to microalgae cell destruction. Zhang et al. [159] eliminated radicals as a possible mechanism since addition of radical scavenger didn't change their results, whereas Wu et al., Wang et al., Batista et al. and Xu et al. [29,49,157,163] ascribed higher cell reductions to more radicals formed. Joyce et al. [3] tried to give a more thorough explanation and tested the same intensities for HFUS and LFUS. They showed that higher frequencies are more effective and concluded that another important mechanism must be taking place at higher frequencies and proposed formation of a bigger number of radicals. Li et al. [17] observed a correlation between the number of free radicals and microalgae destruction, which starts when $^{\bullet}OH$ concentration is above 1 μmol/L and for the first time demonstrated that radicals formed during HC could have effect on microalgae cells. There is however data available in the literature that deals with destruction of microalgae with different oxidants alone. H_2O_2 has been shown to have negative effects on cyanobacteria, algae and diatoms [172–174]. Mikula et al. [173] detected changes in metabolic system, loss of membrane integrity, cell lysis of *M. aeruginosa* when high enough H_2O_2 concentration was used. Drabkova et al. [107] additionally determined the negative effects of H_2O_2 on the photosynthetic system. Gu et al. [175] determined that $^{\bullet}OH$ formed under photocatalysis were responsible for the first radical-initiated changes in the cyanobacteria outer layer, which eventually led to leakage of cell inclusions like chlorophyll *a* and proteins. Bai et al. [176] treated algae *Heterosigma akashiwo* and *Sceletonema costatum* with $^{\bullet}OH$ produced by a novel, very efficient treatment system. When they combined plasma with HC, they observed gaps formed in cell membranes. Since no cellular material leaked from the cells, they speculated that the reason for this is that $^{\bullet}OH$ passed through these gaps into the cells and caused irreversible damage to DNA.

4.5. Effects of cavitation on viruses

As can be seen in the Table 7 there is not much new data available about effects of cavitation on different types of viruses and only one article is available that studied effectiveness of HC.

MS2 seems very susceptible to inactivation with all types of cavitation [2,51,61] regardless of the initial concentration and medium tested only that in the case of higher concentrations more time for

Table 7
Review of cavitation effects on viruses reported in the literature in the last decade.

Species	Characteristic (diameter)	Medium	Conc. PFU/mL	Proposed mechanism	US freq. (kHz)	Effectiveness (%)			Time (min)	Ref.
						LFUS	HFUS	HC		
MS2 bacteriophage	24–27 nm [113]	BS	10^6	/	20	4.62[a]			30[b]	[51]
			10^4			>3.99[a]			10[b]	
		BS	10^3–10^4	/	582		>99		30	[2]
		TW	10^9	M + C	–			4.8[a]	1040p	[61]
			10^3					>2.6[a]	208p	
Feline calicivirus (FCV-F9)	27–40 nm [113]	BS	10^6	/	20	2.67[a]			30[b]	[51]
			10^4			>4.00[a]			5[b]	
Φ X174 bacteriophage	23–27 nm [113]	BS	10^3–10^4	/	582		90		18	[2]
Murine norovirus (MNV-1)	28–35 nm [113]	BS	10^6	/	20	0.07[a]			30[b]	[51]
			10^4			>3.79[a]			30[b]	

LFUS: low frequency ultrasound; HFUS: high frequency ultrasound; HC: hydrodynamic cavitation; BS: buffer solution; TW: tap water; a: log reduction; b: pulsed mode 30 s on 30 s off; p: number of passes (1L samples); /: data not available.

inactivation was needed [51,61]. The same can be deducted in the case of FCV-F9 and ΦX174 [2,51]. MNV-1 also proved susceptible to inactivation but only in low titres [51] and the authors didn't offer any speculations on the reason why. In the case of MS2, Kosel et al. [61] observed that higher inactivation was achieved when bigger volumes were tested (1 L). This was ascribed to development of more aggressive cavitation in the bigger HC design. Since the cloud radius before collapse is larger in bigger scale device more potent pressure pulse is therefore released, which results in greater effect.

The exact mechanism how cavitation causes virus inactivation is not yet elucidated and there is not yet an explicit mechanism (chemical or mechanical) responsible for the observed effects proposed. Su et al. [51] suggested that the damages inflicted during cavitation on the outer protein capsid itself or recognition sites on the capsid surface could be the reason for virus inactivation. Kosel et al. [61] summarized that ˙OH generated during cavitation together with mechanical effects could be held responsible for virus inactivation by affecting viral capsid or genome. There is however a lot of data available in the literature that describes the effects of different oxidants and oxidation treatments (other than cavitation) on different viruses. Cho et al. [112] showed that the inactivation of MS2 phage was the result of OH radicals formed during TiO_2 photocatalysis. The ˙OH caused the denaturation of proteins in the protective capsid. Pottage et al. [114] reported that H_2O_2 vapour very effectively inactivates MS2 and suggested the mechanism behind is the formation of ˙OH which can react with thiol groups present in the viral capsid, lipids and genome. Goyal et al. [177] demonstrated the same effectivity of H_2O_2 for feline calicivirus. Mayer et al. [113] investigated effects of different water treatment processes on several different viruses, including the ones in Table 7. They showed that the amino acid composition of the capsid plays an important role in virus inactivation with oxidative species. They reported that inactivation by ˙OH is correlated with the amount of amino acid tyrosine present in the capsid. To conclude, based on the available literature the composition of the viral outer capsid and the oxidising species in question plays an important role in the pathway of virus inactivation [96,113]. For example, the capsid composition might be responsible for longer inactivation time needed for ΦX1 v74 (hydrophilic capsid) as compared to MS2 (hydrophobic capsid) [2] under the same treatment conditions.

5. Discussion and recommendations

5.1. Why is it so difficult to compare the data from the literature?

5.1.1. Microbiological aspect

In the reviewed literature authors used organisms at different growth phases for their experiments, but not many investigated its effect on cavitation efficiency. Gao et al. [88] for example observed that bacteria are of different shape in different growth phases. They proposed that due to this, bacteria could have different biological and physical properties which may both influence the effect of LFUS on bacteria destruction. However, the same authors showed that the rate of destruction of bacteria is comparable regardless of their growth phase in experiments with HFUS [99]. Similarly, Zhang et al. [159] did not find any effect of algae growth phase using LFUS. In contrast, Balasundaram and Harrison [121] noticed higher protein release in rapidly growing *E. coli*. They summarized that slow growing bacteria could strengthen their cell wall which would result in lower destruction rates.

Normally, microorganisms are incubated in growth medium to promote their growth. On the other side, for cavitation, a non-complex water medium is desired as different mediums can affect the cavitation phenomenon [38,50,51]. Therefore, some authors used distilled or deionized water (i.e. purified water) as treatment medium or as a part of culture preparation [40,86,117,122,123,127,149,178]. Purified water has a negative effect on bacteria since it acts as a hypotonic solution, which leads to cell swelling and possible cell burst. Therefore, it could influence the results [117,160]. It is assumed that medium can affect cavitation phenomenon, whereas Loraine et al. [93] postulated that more complex matrix as sewage water does not affect disinfection by HC.

Another problem arises when authors report results on a different scale – logarithmic or decimal. When inactivation results are presented in percentages, 90% sounds like a high number but it only means 1 log in a log scale. With microorganisms, we are dealing with population numbers up to 108 or even more. Hypothetically, if initial culture concentration is 104 CFU/ml and inactivation rate after treatment is 90%, there are still 103 active cells left in suspension. After microorganism's doubling, the cell number is soon going to be even higher than before treatment and therefore the effect of inactivation is nullified.

To determine the effect of treatment, most authors employed the classical plate count method, which is a retrospective method. Additionally, some bacteria in the environment cannot be cultured but are metabolically active cells (VBNC). For example, pathogens in a VBNC state may remain virulent and produce enterotoxins. Therefore, culture-independent techniques are superior to the plate count method for real-time, quantitative assessment of cell viability and functionality. For example, flow cytometry has greater sensitivity compared to classical plate count method [55,125]. When implementing retrospective methods, it is also important to pay attention of keeping samples on ice to reduce cell activity and therefore minimize differences of handling time.

Even though not many authors in the reviewed literature dedicated much thought to these aspects and others not emphasized in this review paper, they could play an important role in cavitation efficiency. Until the effects of growth phase, cell wall integrity and medium on

susceptibility of microorganisms to destruction with cavitation are unequivocally determined, it is difficult to compare the literature data. Similarly, the same can be said for results presentation and detection methods.

5.1.2. Cavitational aspect

Even though cavitation was first observed on ship propellers in 19th century, it is still not fully understood. Due to its complexity and unpredictable nature, the progress in revealing its behaviour and consequences, is slow. Since the mechanisms, that might affect microorganism's destruction are not known, it is extremely difficult to optimise the cavitation treatment. That all details of cavitation phenomenon are still not completely understood can be seen in the reviewed literature. Most of the authors only report that cavitation is present but do not give any specifics on its characteristics. Not only the dispersity of investigated operating conditions, but also their vague description is the reason for poorly reproducible results. The researchers should also clearly state if the sample is in direct or indirect contact with the surface emitting ultrasound.

One of the parameters, generally not adequately described is the intensity of cavitation. As mentioned in Section 2, there is no consensus on the definition of cavitation intensity yet. Despite that, reporting only the electrical power consumption of the cavitating device does not suffice [6,24,41]. Calorimetry is a good method how to estimate the energy, which is actually delivered to the liquid [3]. However, one must be aware that the properties of the generated cavitation will depend also on the surface area, through which the energy is delivered. If the diameter of the US horn tip or the dimensions of the US bath are missing, the experiments are once more impossible to reproduce.

When describing HC operating conditions, the influence of inlet pressure, flow rate or velocities in the constrictions are often considered as critical factors influencing results. Based on previously mentioned parameters cavitation number is often determined and premature conclusions are made i.e. that the highest removal rate is conditioned by this parameter. Since cavitation number itself only vaguely describes the cavitation characteristics [102], most authors misinterpret the results or draw non-relevant conclusions. One must be aware that cavitation behaviour depends on many mutually influencing operating parameters and that changing one of them, will also influence all other parameters. Thus, concluding that one specific parameter influences the results might be misleading. Additionally, by not determining the actual power consumption of cavitating set-ups (i.e. pump together with RS devices) but reporting only the nominal electrical power leads to mis conclusions of energy efficiency of the system. Based on the emphasized aspects the conclusions from the reviewed literature are difficult to compare.

5.1.3. Chemical aspect

As discussed in Section 4 different cavitational conditions (i.e. cavitation type, cavitation device and sample treated), can lead to formation of different amounts of reactive species. The pathway of reactive species formation during cavitation is very complex which is implicitly presented in Gągol et al. [98]. Similarly, that different oxidants can provoke various outcomes in microorganisms is discussed in Wigginton et al. [96]. In addition, it should also be taken into consideration that microorganisms can defend themselves against these species but only to a certain degree. Once their defence mechanisms are depleted oxidative stress ensues. It is for example postulated that nutrients present in microorganism's growth medium could influence their defence mechanisms [130]. Additionally, Vázquez et al. [179] reported that "domesticated" laboratory strains compared to environmental strains proved more resistant to oxidative stress. In addition, Gao et al. [99] noticed that radicals formed during cavitation decreased the pH of the solution, which could consequently inactivate investigated microorganisms. Thus, it is evident that a more systematic investigation on the effects of oxidants on different microorganisms must be performed. It should however be emphasized that even though above-mentioned considerations are taken into account there will always be something else to consider.

Therefore, currently it is hard to compare the available literature since these considerations are not taken into account by most authors. Likewise, it is impossible to conclude whether reactive species induced by cavitation have any effect on microorganism's destruction based on the reviewed literature. A lot of the authors only assumed that radicals could be responsible for microorganism's destruction and cited previous literature but did not corroborated their assumptions in any way (for example by addition of radical scavenger).

5.2. Recommendations and the way forward

- Even though not many authors of the reviewed literature tackle the topic of microorganism's growth phase we recommend that in the future the investigations should be done on microorganisms in stationary phase. In this manner it will be much easier to compare the results and determine if and how growth phase influences effect of cavitation.
- Similarly, to more easily compare the results we recommend that presenting results in logarithmic values is more appropriate.
- Considering the aspect of cavitation, the researchers should report the accurate description of the cavitating geometry. In addition, when possible, images of the cavitation should be provided. These should be, whenever possible, accompanied by measurements of pressure fluctuations which should be reported.
- We observed that many authors use cavitation number as the key parameter, that vaguely describes the cavitation stage. We recommend that together with this they should also give the precise location of pressure measurements, the flow rate, the position of the velocity measurement and the medium temperature (as it was already called upon by Šarc et al. [102]).
- Additionally, the specifics of the medium characteristics, such as pre-treatment (i.e. filtration, deionisation and distillation), the gas and solid particle content, should accompany every report.
- To determine the role of reactive species we recommend that first it should be unequivocally determined whether reactive species are generated during cavitation. Since microorganisms can tolerate oxidative stress to a certain degree, it should be determined which reactive species and in what amount are generated. Only then it would be prudent to determine whether formed species are responsible for destruction of microorganisms.
- The most important oxidants that should always be determined, if authors want to suggest that reactive species are the reason for microorganism's destruction, are H_2O_2 and $^{\bullet}OH$. There are numerous different determination methods already described in detail in the literature. Once this is determined also more specific effects of how radicals affect microorganisms should be elucidated by studying which cell constituent is affected (i.e. cell wall and/or intracellular components).

6. Conclusions

As it can be seen from the summarized literature data there are a lot of inconsistencies. It is evident that most of the research is focused on AC and that investigations on the potential of HC for microorganism's destruction have only recently gained more attention. It is also evident that there has not been much progress in terms of developing new ways of cavitation generation especially in the case of HC.

The biggest problem is that most authors only cite previous assumptions regarding cavitation's mechanisms of action and they neither investigate nor offer additional and corroborated new possibilities.

There is however a consensus that a method that would be able to destroy microorganisms in environmental samples or food industry is needed. Cavitation may prove to be such a method and one that could

satisfy all requirements – effectively and relatively quickly destroy microorganisms without any collateral damage such as production of secondary pollutants. But in order to use it most efficiently (to exploit it to its highest potential) the exact mechanisms by which it interacts with microorganisms must be elucidated. This can only be done if scientists from different scientific fields join forces – and this is what ERC project CABUM will focus on in the next 5 years.

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Appendix A. Supplementary data

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