Chemical Engineering Journal 382 (2020) 122383

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Synergistic effects of trace concentrations of hydrogen peroxide used in a novel hydrodynamic cavitation device allows for selective removal of cyanobacteria

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HIGHLIGHTS

- New hydrodynamic cavitation device creating multiple cavitation clouds was developed.
- Precise adjustability allows selective removal of cyanobacteria while leaving algae undamaged.
- Hydrogen peroxide addition enhances the lethal effect to cyanobacteria.
- Wide applicability for enhanced technologies in homogenisation, mixing and oxidation processes.

ARTICLE INFO

Keywords:
Cyanobacterial blooms
Algae
Water treatment
Chemical technology
Hydrodynamic cavitation
Hydrogen peroxide

ABSTRACT

Here, we present an improved and verified rotating hydrodynamic cavitation device (RHCD) inspired by so-called cavitation heaters. The cavitation efficiency of the device is adjustable by rotation speed and flow rate and can be modified for selective removal of cyanobacteria from water with only temporal effect on algal growth or metabolic activity. Previous hydrodynamic cavitation devices have required several cycles to achieve cyanobacterial elimination (12–200 cycles, 5–200 min of treatment), while the RHCD is capable to remove 99% of cyanobacteria after a single cycle lasting 6 s. The device efficiency at cyanobacterial removal was synergistically enhanced through the addition of trace concentrations of hydrogen peroxide (45–100 μM H_2O_2), levels 10–1000 times lower than those used in previous studies. The RHCD is also capable to increase temperature, an additional advantage for potential technological applications. We discuss the potential use of this device over a broad spectrum of technological processes, and especially regarding the addition of hydrogen peroxide, ozone, or ferrates, which could open new areas in advanced oxidation technologies. It could also be used as an alternative or as a complement to sonochemical, microwave-assisted or electrochemical methods in chemical engineering processes requiring treatment of large volumes of liquids.

1. Introduction

Cyanobacterial blooms and their negative impacts on aquatic ecosystems are increasing around the globe. Excessive growth of cyanobacterial biomass has been linked to nutrient enrichment of waters (mainly by phosphorus and nitrogen), increasing water temperature and increased level of carbon dioxide [1]. These impacts result in the degradation of aquatic ecosystem stability, along with decreased biodiversity, loss of fish production and recreational value and reduced availability of drinking quality water [2]. The serious economic, cultural, ecological, health and social problems related to cyanobacterial blooms around the world [3] prompted new technologies for the prevention, mitigation or removal of cyanobacterial biomass and their associated toxins from surface, recreational and drinking waters. A primary method for preventing cyanobacterial blooms is to increase aquatic ecosystem stability through the prevention of nutrient enrichment by dissolved phosphorus and nitrogen. Though a widely accepted requirement for sustainable aquatic management, there is a number of cases where practical and economic constraints make it neither feasible nor effective to work with nutrient levels only. For drinking water production, fish production and recreational waters bodies in particular, there is an increasingly urgent requirement for alternative tools for cyanobacterial bloom management. Numerous scientific studies, industrial patents and even commercial applications now claim to

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https://doi.org/10.1016/j.cej.2019.122383
Received 14 May 2019; Received in revised form 26 July 2019; Accepted 29 July 2019
Available online 30 July 2019
1385-8947/

provide effective and fast-acting methods for their removal; however, critical approaches need to be applied in order to distinguish laboratory experiments, ecosystem or mesocosm experiments and up-scaled technological applications. A broad spectrum of methodologies are now available, including application of algaecides or selectively acting cyanocides [4], biologically derived extracts [5], barley extracts [6,7], decomposed litter extracts [8] and allelopatic interaction [9]. In addition to the biological and chemical methods for cyanobacterial bloom management, there is a number of mechanical technologies available, including sediment dredging, destratification and artificial mixing [10,11] and a broad spectrum of physical methods including strong ionisation discharge [12], high voltage pulse discharge [13], and a diverse group of acoustic (ultrasound) cavitation or hydrodynamic cavitation (HC) methods. However, not all of these technologies are fully accepted; ultrasound treatment, for example, being strongly recommended by some [14,15] and strongly warned against by others [16].

Hydrodynamic cavitation as the technology for water treatment became to be more and more published alternative to standard methods, however, published data are based predominantly on laboratory and small-scale devices [17–19] and requires long-term contact time [20,21]. Comprehensive actual review of the wastewater treatment based on cavitation was published by Gagol et al. [22]. They shows, that in 2006 it was published 8 papers concerning hydrodynamic cavitation whereas 10 years later in 2016 118 papers was published on this topic and number is still increasing. This lucid review proved, that hydrodynamic cavitation can be used for removal of toxic pollutants or pathogenic microorganisms. Different constructions can be used for hydrodynamic cavitation devices and include Venturi tube, orifice plates, or rotation devices. Hydrodynamic cavitation can be combined as an advantage with different advanced oxygenation technologies, what is actually the main stream not only for wastewater, but also for drinking water treatment [23–25]. Increasing attention is dedicated also to the economy and energetic consequence of hydrodynamic cavitation. Comparing the acoustic cavitation, Venturi tube, orifice plates and rotation devices for water disinfection, the last ones seem to be effective [26], but a new devices for hydrodynamic cavitation using vortex diodes were recently published [27] so new development in this area can be expected soon.

In this study, we present a novel HC device with parameters important for its practical application as regards non-chemical treatment of surface and technological waters, which novelty is focussed especially on parameters important for practical application i.e. the ability to remove cyanobacterial biomass from the water column, low number of cycles needed, minimal working pressure and low operational cost.

2. Material and methods

2.1. Experimental setup

The modified and improved rotating hydrodynamic cavitation device (RHCD) was inspired by cavitation heaters developed for heating liquids through mechanical processes (inspired by an old US patent (US5188090A), but was modified to enhance the generation of cavitation clouds and improve the rotor pumping capacity). The device consists of a rotor represented by a thick disc (diameter 160 mm, thickness 86 mm) with 162 holes drilled around its perimeter (diameter 12 mm, depth 20 mm), their axes being inclined slightly backward (see Fig. 1). During rotation, cavitation clouds develop within vortices that fill the holes and the gap between the rotor and stator. Water is supplied from a pressurised vessel, an external compressed air source being used to maintain sufficient pressure (see Fig. 2).

RHCD inlet and outlet pressure was measured using DMP331 pressure sensors (BD Sensors), while flow rate was measured using the IMQI99-SN induction flowmeter (ELA, range 0–0.8 $L\,s^{-1}$, accuracy ± 0.5% of range). Inlet and outlet temperatures were monitored using HSO-502 1A2L probes. Experimental conditions were as follows: 4000–5000 RPM pressure 105–265 kPa, single treatment (1 cycle), working volume 20–250 L, flow up to 0.3 $L\,s^{-1}$, three replicates. During operation, pH and conductivity remained unchanged (conductivity 0.45–0.47 $mS\,cm^{-1}$ and pH 7.12–7.43). Temperature was controlled during the treatment by regulating water flow speed (flow rates was kept between 0.12 $L\,s^{-1}$ and 0.3 $L\,s^{-1}$ to keep the temperature below 26 °C).

2.2. Cyanobacterial biomass

Fresh cyanobacterial biomass was collected from the Brno reservoir 24 h before the cavitation experiments. The biomass was filtrated in order to remove solid impurities > 1 mm and biomass density was increased by filtering through a 0.25 mm mesh plankton net. Microscopic determination indicated that 95% of the biomass consisted of *Microcystis* sp. (*M. aeruginosa, M. viridis, M. flos aquae, M. wesenbergii*). The biomass suspension was then homogenised, gently aerated and enriched with Z-medium.

2.3. Chlorophyll fluorescence measurements

Chlorophyll fluorescence was assessed for both control and treated cyanobacterial suspensions using an AquaPen AP 100-C fluorometer (PSI, Drásov, Czech Republic) with cuvette holder within two hours of treatment, and again after 24 and 48 h. The fluorometer was set to the amber-red (620 nm) light source for measuring, with actinic and saturation lights. All samples were pre-adapted in darkness for 10 min at room temperature (22 ± 1 °C).

2.4. Growth inhibition test

All experimental treatments and controls were transferred into a 96-well plate (250 µL per well) within two hours of cavitation treatment and kept for 96 h in a cultivation room at 22 ± 2 °C and continuously irradiated at 100 $\mu mol\,m^{-2}\,s^{-1}$ under 36 W warm-white fluorescent tubes (Kanlux, Czech Republic). Chlorophyll concentrations were then measured every 24 h using a Genios fluorescence microplate reader (Tecan, Austria) with excitation set at 590 nm and emission at 680 nm.

2.5. Hydrogen peroxide pre-treatment and detection

Hydrogen peroxide (H_2O_2; Sigma, USA) treatment (10, 20 or 30 µL of 30% H_2O_2 per 10 L) was applied prior to filling the (RHCD) and immediately exposed to cavitation treatment. Immediately after, 4 mL aliquots were filtered through an ultrafine syringe filter (0.45 µm pore size) and mixed with a detection solution containing titanyl ions (Sigma, USA), absorbance being measured spectrophotometrically at 407 nm.

2.6. Statistical analysis

All tests were run at least three times. Following verification of normal distribution and variance homogeneity, the data were processed using analysis of variance (ANOVA), with differences being compared using the Tukey HSD range test with significance set at $P < 0.05$.

3. Results and discussion

Two types of cavitating structures arise in the RHCD. First there are cavitating vortices arising from the holes on the rotating disc perimeter. Second, it is cavitation due to the shear forces in the thin gap between the case and rotating disc. Both principles contribute to the removal of cyanobacteria, inhibition of photosynthesis and disintegration of their colonies. Rotational forces ranging from 1000 to 6000 RPM were tested during the preliminary experiments, and values 4500 and 5000 RPM

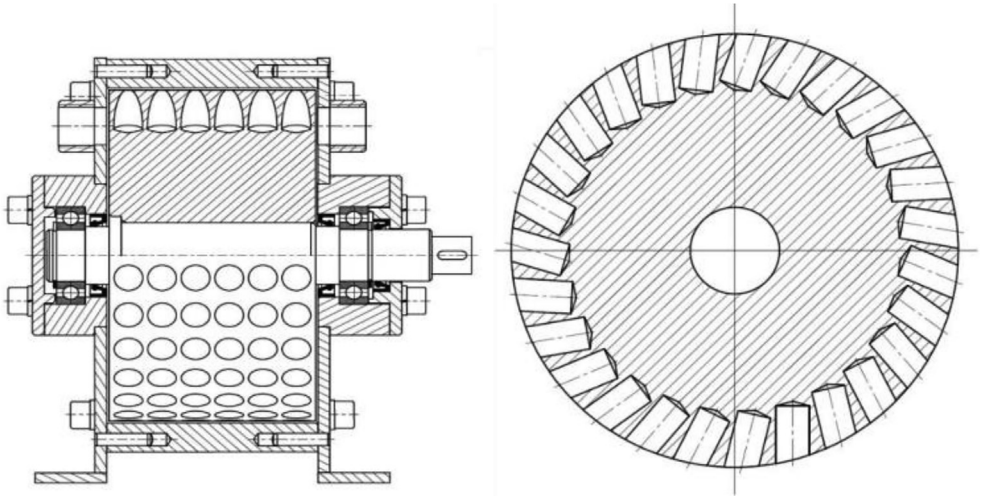

Fig. 1. Schematic layout of the rotating hydrodynamic cavitation device (RHCD).

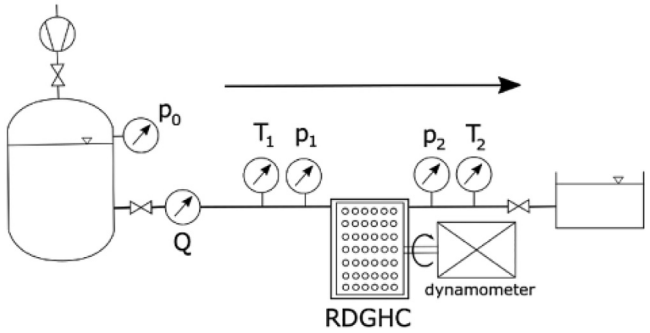

Fig. 2. Schematic layout of the experimental circuit.

were finally selected as optimal for the experiments with cyanobacteria. Rotation speeds above 6000 RPM increased significantly the temperature (by up to 20 °C after a single flow) and appear promising for technologies where increasing temperature is an advantage, e.g. process intensification in extraction, depolymerisation, proper mixing of liquids or liquid-gas, liquid-solid particles, emulsification etc. Recent review of cavitationally driven transformations as a technique for process intensification [28] mentioned also crystallization, wastewater treatment, or water disinfection, what is also a topic of our paper concerning cyanobacteria removal from water.

While growth and photosynthesis of *Microcystis* sp. in the control was exponential, all cavitation treatments resulted in inhibition of cyanobacterial growth and photosynthesis (Figs. 3 and 4). Cavitation at 4500 RPM itself and H_2O_2 treatment alone resulted in < 40% decline of cyanobacterial biomass and activity, a combination of 5000 RPM and

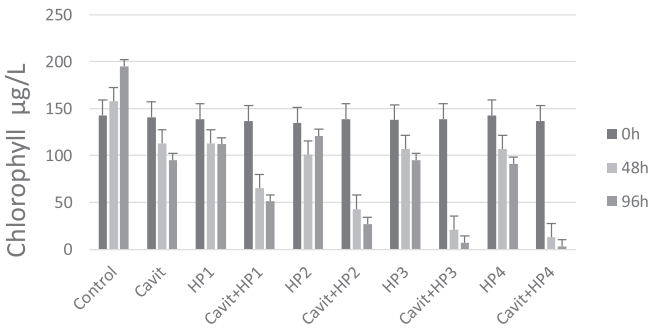

Fig. 3. Growth of *Microcystis* sp. at two and four days after cavitation at 5000 RPM in combination with H_2O_2 at 1, 2, 3 and 4 µL L^{-1} (HP 1, 2, 3, 4).

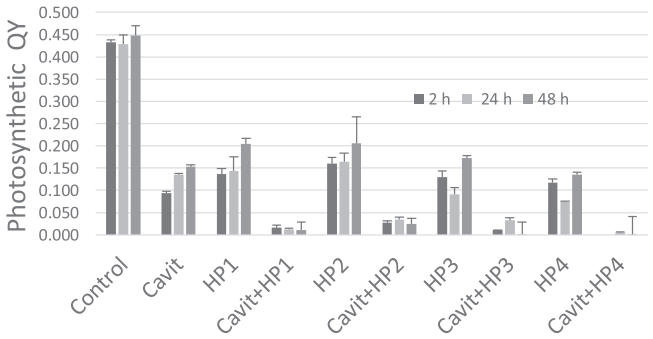

Fig. 4. Photosynthetic activity of *Microcystis* sp. following cavitation treatment at 5000 RPM in combination with H_2O_2 at 1, 2, 3 and 4 µL L^{-1} (HP 1, 2, 3, 4).

H_2O_2 treatment achieved removal rates of 75, 87, 97 and 99% in the case of 1, 2, 3 and 4 µL L^{-1} H_2O_2, respectively (Figs. 3 and 4). The H_2O_2 concentrations used in this study were 6–18 times lower than those used in [18], and 200–4000 times lower than those in [23]. The trace concentrations of H_2O_2 used in this study proved effective as synergic effects with the rotating device resulted in HC. This was especially true at 5000 RPM combined with 3 or 4 µL L^{-1} H_2O_2, which resulted in a 97 and 99% reduction in cyanobacterial growth and 98 and 99% inhibition of *Microcystis* sp. photosynthesis, respectively (Figs. 3 and 4). Fig. 5 present effect on the biomass of *Microcystis*, where differences are not clear immediately from the graph, but the calculation of synergic effects with the biomass data are in Table 3 in SI where we can see, that in the case of single treatment by the hydrogen peroxide 3 µL L^{-1}, the

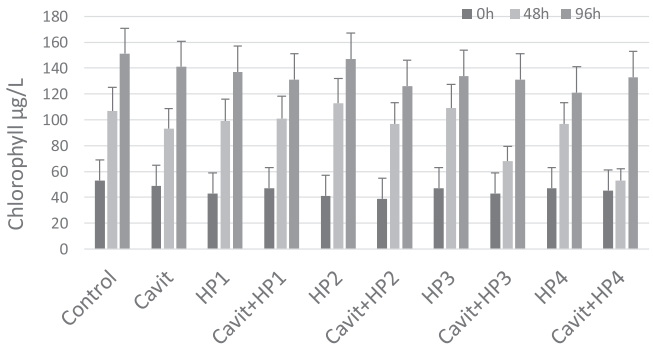

Fig. 5. Growth of the green alga *Desmodesmus quadricauda* at two and four days after cavitation at 5000 RPM in combination with H_2O_2 at 1, 2, 3 and 4 µL L^{-1} (HP 1, 2, 3, 4).

inhibition of biomass was 32%, cavitation single treatment inhibited the biomass of *Microcystis* by 28%, so simple sum is 60% of inhibition, but combination of HP and HC inhibited the biomass of *Microcystis* by 86.7%, what is 26% synergic effect. Similarly we can see in the case of HP 4 μL L^{-1} - single effect is 33% of inhibition, single cavitation produce 28% of inhibition, what is in sum 61%, but combination of 4 μL L^{-1} of HP and 5000 RPM of HC produced 91.8% of inhibition, what is 30% more that any single treatment.

The speed and forces producing cavitation in the RHCD can be adjusted to produce selective removal of cyanobacteria (Fig. 5), with no relevant effects on algal growth (*Desmodesmus quadricauda*) detected. This selective removal is highly important from practical point of view, because algae in phytoplankton did not produce toxins or odours like cyanobacteria, which are problematic organisms in drinking and recreational waters. That is why the selective removal of cyanobacteria is important more than total phytoplankton removal, because algae can keep the role of primary producers in utilise nutrients replacing the cyanobacteria which are hygienically and technologically problematic organisms.

Likewise, analysis of algal photosynthesis showed no effect of cavitation, H_2O_2 or their combination (see SI Figs. 1 and 2), thereby supporting the use of the RHCD as a selective tool for removal of cyanobacteria. The devices adjustability and selectivity would also be a strong advantage in water treatment, where cyanobacterial cells with reduced photosynthetic activity and collapsed gas vesicles are more easily separable than floating cells and colonies.

Microscopic observations showed that cavitation treatment resulted in the destruction and disintegration of *Microcystis* colonies and cell gas vesicles, though the cell structure was not destroyed (Fig. 6). This ability for adjustable destruction would be a great advantage in drinking water treatment, where removal of cyanobacteria from the water column could be achieved without chemical pre-treatment and without leakage of cyanotoxins and organic compounds from cytoplasm into drinking water. Sensitive adjustment can cause also only slight injury like gas vesicle destruction without cell lyse, what provide 25–40 h of sedimentation but later gas vesicle can be synthetized (see SI Fig. 3).

Hydrodynamic cavitation is produced in a special geometry by mechanical rotation of an object through a liquid at a specific flow velocity. Combination of kinetic energy and high pressure due to unique geometry of RHCD creates hydrodynamic cavities with high cavitation energy. Processes, which are involved in the observed effects on the photosynthesis and disintegration of colonies and cell viability of *Microcystis* include i) the growth and quick collapse of cavities in the device, where temperature can reach for a few microseconds 1200–1500 K, ii) extremely high velocity in the centre of cavities reach 120 m s^{-1} what can not only disintegrate colonies, but also injure subcellular structure of *Microcystis*, iii) bubbles collapsing near walls or in the neighbourhood of another cavities adopt non-spherical shapes, which cause the ejection of high speed micro-jets with diameters of a few microns; such extreme conditions are able to rupture or kill biological structures or iv) they can cause water molecules to dissociate into oxygen radicals (the trace concentrations of hydroxyl radicals produced by RHCD was also measured and will be a subject for further study), iiv) strong shearing forces can support the mechanical injury of *Microcystis* cells and in this experimental design they strongly improve the mixing of trace concentrations of hydrogen peroxide. All these principles and processes should be involved in the observed synergic effects and altogether creates this device perspective for water treatment processes.

A comparison of basic parameters used in different HC devices for water treatment and cyanobacterial removal (Table 1) showed that our device was larger-scale, worked at lower pressure, needed just one cycle (compared with 90, 150 or 181 cycles) and needed a much shorter treatment time (0.1–0.3 min compared with 10, 45, 60 or 150 min). Further, effectivity of cyanobacterial biomass removal was 97 up 99%, in comparison to 88% after 106 cycles and 10 min of cavitation [17], or 61% removal of cyanobacteria after 188 cycles, 10 min treatment with ozone [18].

In general, rotating devices are 10–100 times more effective at HC generation than orifice or Venturi devices [26]. For example, the orifice device described in [29] had a pressure of 31 MPa, a 150 min reaction time, addition of 0.4 M H_2O_2 with adjustment to pH 2.5 needed, compared with the 105 kPa, 0.1 min reaction time and 0.1 H_2O_2 mM used in this paper. RHCDs are perspective as a multitude of force effects act on treated component mixtures due to the collapse of cavitation bubbles. New developments in this field offer not only simple heating for disinfection [30] but also precise use of HC variables [31].

Both the novelty and effectiveness of our RHCD suggest its potential use in a number of industrial applications, particularly as we only used a part of its potential range of volume, rotation speeds, pressure, temperature and other parameters. It should prove especially useful in water treatment and chemical technologies where mixing of even trace reagents, quick reaction time and/or enhanced temperature (with no external heating source) are required. The particular advantages of our RHCD could be further exploited in technologies where advanced oxidation methods are applied as our preliminary data indicated that the RHCD produced hydroxyl radicals. This could prove especially useful in combination with other advanced oxidation processes and green-chemistry technologies [32,33], offering a broad spectrum of

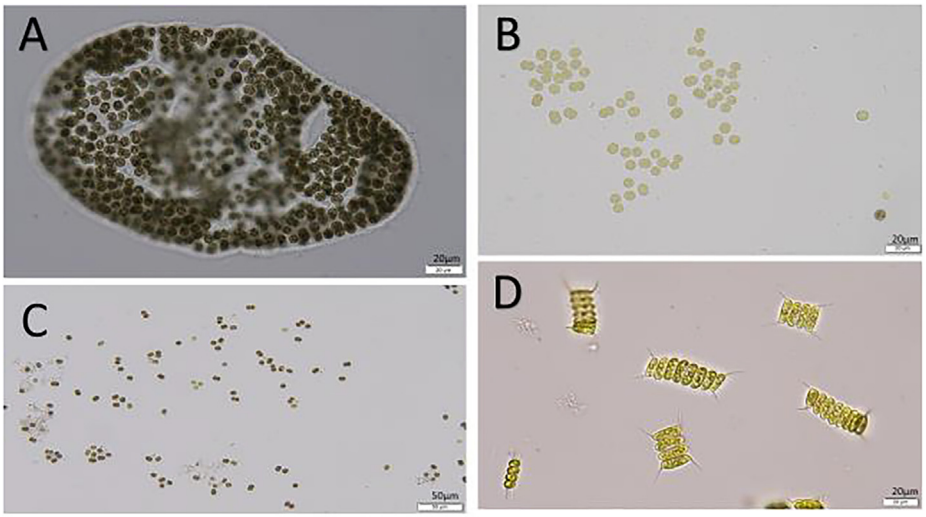

Fig. 6. Microphotographic documentation of cavitation response. A) *M. aeruginosa* – control. B) *M. aeruginosa* – 96 h after cavitation at 5000 RPM and 4 μL L^{-1} H_2O_2 treatment – note intact cells but without gas vesicles. C) 96-hours after cavitation at 5000 RPM without H_2O_2 treatment – note disintegrated colonies but black dots inside cells indicating new gas vesicle synthesis. D) *Desmodesmus quadricauda* 96-hours after cavitation at 5000 RPM and 4 μL L^{-1} H_2O_2 treatment – note intact and actively growing cells.

Table 1
Comparison of different hydrodynamic cavitation methods used for destruction of cyanobacteria and water treatment.

Cavitation by	Experimental volume (L)	No. cycles	Pressure (kPa)	Treatment time (min)	Note	Removal Rate %	Source
Orifice plate	1.75	181	118–284	10 min	Ozone + HC	24–61	[18]
Orifice valve	5	106	200–500	10 min	600 μM H_2O_2	63–88	[17]
Venturi nozzle	3–10	2–18	350	0.2–3 min	*Microcystis*	90–99	[19]
Venturi	4.5	150–188	270–540	60 min	*Scenedesmus*	33–85	[20]
Orifice plates	1.8–20	90–180	2.1–8.3	45–60 min	*E. coli, Pseudomonas* sp.	30–55	[21]
Rotating	0.25–0.5	continual	Not known	8–20 min	*E. coli, E. faecalis*	99–100%	[26]
Orifice plate	2, 5–8	Not known	31,000	150 min	0.4 M H_2O_2, NZVI TOC removal waste water	50–60%	[32]
Rotating	60	4	70–150	12–20 min	Heating up 70 °C *E. coli*	100%	[30]
Rotating	20–250	1	105–265	10–12 s	45–100 μM H_2O_2	97–99	This paper

applications in microorganism and micro-pollutant treatment in water treatment and technological processes.

4. Conclusion

This paper documents the development and verification of a novel rotational device that generates strong hydrodynamic cavitation for selective cyanobacterial removal from water. Fully adjustable, the rotational device is capable of selectively removing cyanobacteria with no reduction in algal growth or photosynthetic activity. A rotation speed of 5000 RPM and the addition of 0.1 mM H_2O_2 removes 99% of cyanobacterial biomass from the water column in just 10 s through colony disintegration and gas vesicle destruction, and causes 98% inhibition of photosynthesis with no cell destruction, which is of great importance for drinking water treatment technologies. The device is at least 100 times more cost- and time-effective than previously published hydrodynamic cavitation devices for removal of cyanobacteria. Adjustability of pressure, flow speed and temperature, along with its strong mixing ability, make this device ideal for a broad spectrum of technological applications in the water treatment and chemical engineering.

Acknowledgement

This study was supported by the Czech Science Foundation GACR, Grant No. 16-18316S, Czech republic. English corrections and editing was kindly realised by Dr. Kevin Roche.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2019.122383.

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