**Open access readme for:**

**Towards decentralized nitrogen fixation using pulsed ultrasound**

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**Methods**

**The experimental setup**

The ultrasound source was a curved focused transducer (H-149, Sonic Concepts, Bothwell, WA), driven at *f*0 = 200 kHz through a 400 W power amplifier (Electronics & Innovation, Model 1040L). The focused transducer (see Supplemental Video S1 for its simulated profile), the sonoreactor (which is made of quartz glass of diameter 10 mm-Ø, length 130 mm, and thickness of 1 mm), and shock wave passive cavitation detector (swPCD)32 were arranged within a customized tank measuring 420 × 438 × 220 mm3 as shown in Fig. 3. The reactor was positioned at the transducer’s focus, which was 68 ± 1 mm away from the transducer’s front face. The shock wave passive cavitation detector was positioned behind the reactor at a fixed distance of ̴ 30 mm, on the axis of ultrasound propagation.

The tank was filled with ~19 L of degassed water so that the bulk temperature would not deviate significantly from room temperature (~25 °C) during sonication. Before the initialization of each sonication, 6 mL of deionized water was placed in the reactor and sparged with air from a 100 kg cylinder (BOC, Linde Group) for 10 minutes at a constant rate of 19 L/min, which was measured using an in-line flow meter (Masterflex PMR1-010531). A sintered disc with a porosity size of 3 was fitted at the reactor’s base to ensure that the sparged air was split inside the reactor to avoid attenuation of the irradiated sound wave. The focused transducer was driven with both continuous wave (where the transducer is energised continuously for a specified period without pulsing) and pulse width-modulated sine wave signals at amplitudes of 500mVpp, 800 mVpp, and 1000 mVpp through a programmed signal generator (RIGOL DG 4102), RF Amplifier 400 W 10 kHz - 5 MHz 55 dB (E&I 1040L), and a digital delay generator (DG535, Stanford Research Systems, Sunnyvale USA), for various electrical input protocols based on a constant energy scheme. It should be noted that for all the experiments reported in this work, unless specified otherwise, air was continuously sparged into the samples during ultrasound irradiation.

**Acoustic data collection**

The shock wave passive cavitation detector, a custom designed in-house fabricated device, was used to collect the acoustic data. The shock wave passive cavitation detector was constructed from a 15 mm-diameter disc of 110 μm-thick polyvinylidene fluoride (PVdF) film with a thick tungsten epoxy backing layer to minimize resonance. Along with device resilience, an epoxy matching layer offers sensitivity above instrumental noise up to 4 MHz, which decreases with increasing frequency. Interested readers are referred to reference32 for a detailed characterization of the performance of the shock wave passive cavitation detector. For the current paper, the shock wave passive cavitation detector was fixed at a distance of around 30 mm behind the reactor on the focal axis. The acoustic data were collected through an oscilloscope (Tektronix 5 series, Berkshire UK) at 12.5 million samples per second. With the aid of Matlab scripts (MATLAB, MathWorks), a filtering protocol was used to reduce noise outside the useful bandwidth of the shock wave passive cavitation detector, and after the fundamental frequency (*f*0 = 200 kHz) and harmonics (n*f*0) had been suppressed, the shock wave content was revealed for presentation in the voltage time domain, from which the root mean square of the voltage, Vrms, was obtained. The details of the filtering protocols are given in the Supplemental Methods. The Vrms was taken to quantify the time averaged shock wave content within the collected acoustic emission.39 For each input protocol used, three repeats were collected, and the mean and standard deviation of all the data collected were taken.

**High-speed imaging**

High-speed imaging of the cavitation activity was undertaken with a high-speed camera, Fastcam SA-Z 2100 K (Photron, Bucks UK, 128 GB of RAM), recording at 20,000 frames per second (fps), with a 159 ns shutter time, through a macro-lens (EX DG, 24-70 mm 1:2.8, Sigma, Hertfordshire UK) and a 36 mm extension tube. At this frame rate, imaging was achieved over 768 × 768 pixels with a resolution of 20 μm per pixel. The high-speed camera and the oscilloscope were systematically triggered simultaneously with the help of the delay generator (DG535, Stanford Research Systems, Sunnyvale USA). It should be noted that only representative images were collected during selected pulses of ultrasound irradiation due to limited image data storage capacity.

**Nitrate and nitrite assays**

Nitrite (NO2−) was detected and quantified spectrophotometrically using the Griess test.40 In a vial, 1 mL of 6 N of hydrochloric acid (HCl) was added to 5 mL of *p*-amino sulfonamide and the solution was agitated for a minute, after which the solution was then added to 5 mL of the test sample in another vial. After 2 minutes, 1 mL of the coupling agent solution (3.86 mM of *N*-(1-naphthyl)ethylenediamine) was then added to the sample/HCl/sulfonamide solution. The pink color was then allowed to develop for 20 minutes, after which the absorbance was measured at 540 nm using an Agilent Cary 60 UV-vis spectrometer. The mean values of the absorbance from three repeats were compared against the standard calibration curve (Supplemental Fig. S1). Ultraviolet spectrophotometric screening also allowed ready determination of the nitrate (NO3−) concentration after acidification of reaction samples with 1 N HCl and analysis of the absorption at 220 nm.41 Corresponding calibration curves are shown in Supplemental Fig. S2.

Ion chromatography (Dionex ICS 900) was also employed for detection of both nitrate and nitrite.42 A solution of the eluent (mobile phase), specifically a combination of 0.8 mM sodium hydrogen carbonate and 4.5 mM sodium carbonate was prepared, and an anion-exchange column (AG23 Guard column and AS23 Anion column) suitable for nitrate/nitrite analysis was installed. The samples were analyzed on the same day they were prepared without further dilution. 0.5 mL of the samples were injected into 0.5 mL AS-DV autosampler PolyVials with filter caps (Dionex). Subsequently, the samples were analyzed under optimized method parameters, including a flow rate of 0.25 mL/min, at room temperature (25 °C), and an eluent concentration of 0.8 mM. The system was flushed with milliQ water every five samples. The detector response was continuously monitored to identify the peaks corresponding to nitrate and nitrite. The concentrations of nitrate and nitrite were then determined with the help of a standard calibration curve using the Chromeleon software. For the calibration curves, anion standard solutions (Merck, 89886) and nitrite standard solutions (Merck, 67276) were diluted as appropriate with milliQ water.

The nitrate content of the samples was also examined using a commercial combination ion selective electrode (SENTEK).

**Optimization Procedure**

A set of input protocols, based on the same total sonication time (20 s), was tested using different pulse durations and pulse intervals (the time interval between pulses), as shown in Table S4. Various pulse durations were employed while keeping the total sonication time constant (20 s) for each input protocol. To maintain the same input energy to the system at a constant pulse amplitude, the total excitation time of the transducer had to remain the same. Consequently, a larger number of pulses were applied in cases where the pulse duration was short (i.e., a greater number of short-duration “bursts” of sonication were used, ensuring that pulse duration × number of bursts always equalled 20 s). A pulse amplitude of 1000 mVpp was maintained for all experimental runs with the focused transducer. For each pulse duration, five different pulse intervals were tested (20, 40, 80, 120, and 160 ms).

**Home-made prototype device**

The prototype device was made from steel, using a commercially-available 40 kHz, 50 W transducer purchased from Allendale Ultrasonics. This transducer had a shape akin to a truncated cone, with dimensions 54 × 45 × 35 mm for the height, diameter of the base, and diameter of the top face respectively. The sonoreactor working chamber was designed with a total internal volume of 100 mL (see Fig. S8 for details of the dimensions of the sonoreactor). The acoustic power density for the device was estimated calorimetrically to be 33.5 ± 0.8 W/L

For each experiment conducted with this device, 20 mL of sample was placed in the device. The device was operated using a commercially available Piezo Driver (PDUS210 Ultrasonic Driver) at an electrical input power of 150 W, in both continuous mode and pulse mode (pulse duration = 4 ms, pulse interval = 80 ms).

**Supplemental Note S1: The Birkeland-Eyde process**

The Birkeland-Eyde process uses electrical arc discharging to produce NO from N2 and O2 at temperatures of 3000 °C. The NO subsequently reacts with O2 as it cools, producing NO2 (nitrogen dioxide), which further reacts with water to produce nitrate (NO3⁻). The Birkeland-Eyde process has the distinct advantage that it does not require the separation of N2 from air (or indeed the production of H2 as with the Haber Bosch process) prior to the fixation reaction. Despite this, the Birkeland-Eyde process still requires large facilities and surrounding infrastructure to operate and although this is not employed industrially at the current time.S1

**Supplemental Note S2: Process of ultrasound induced bubble formation**

Ultrasound is a sound wave beyond the human hearing and thus behave as every sound wave. It consist of the compression and rarefaction phase. During sample irradiation with ultrasound, the rarefaction cycles exert negative pressures on the liquid, pulling the molecules away from one another. If this negative pressure is large enough, then the rarefaction cycle can create cavities (bubbles) in the liquidS2 that oscillate in response to the pressure fluctuations of the ensuing sonication, which depend on the amplitude and frequency of the acoustic field. Phenomena such as rectified diffusion, bubble fragmentation and bubble coalescence occur during the oscillation phases. Throughout this process, heat is generated due to the cyclical adiabatic compression of the gases contained within the bubbles. Eventually, this heat is released when the bubbles collapse. In addition to high temperatures, pressure spikes (shock waves) of up to 1 GPa,S3 plasmas, light (sonoluminescence), and liquid jets are also produced. The latter occur when the bubbles collapse asymmetrically or against an obstacle.S4 In general, cavitation is a nucleated process, meaning that it originates from a pre-existing “weak point” in the liquid, caused by impurities/contaminants, dissolved gas, or gas pockets,S5 such that the onset of cavitation requires much lower amplitudes/intensities than would otherwise be expected for pure, degassed liquids. Aerated aqueous solutions (i.e., solutions sparged with air) therefore provide an ideal medium for inducing cavitation events under an applied ultrasonic field.

**Supplemental Methods**

**Acoustic emission and filtering procedures**

Acoustic emission is the secondary sound waves emitted by the cavitating bubbles. These acoustic emissions can be detected using sensors like hydrophones and passive cavitation detectors. Careful and detailed analysis of the detected acoustic emission can reveal useful information about cavitation activity.S6,S7

In cavitation-mediated applications, the detected acoustic emissions are often correlated with their effects for control and optimization purposes. For example, in certain medical therapy applications, a specific spectral component of cavitation serves as a reference point for monitoring and controlling cavitationS8. In industrial applications, the average power within the sensor’s active bandwidth is utilized as a cavitation index to estimate the intensity of the generated cavitationS6,S7,S9,S10,S11.

In this study, we have used acoustic emission detected with a bespoke shock wave passive cavitation detector (referenced in the main manuscript). The acoustic emission was post-processed via filtering protocols as follows:

The acoustic emission was first filtered from the noise outside the sensitive bandwidth of the shock wave passive cavitation detector sensor used (400 kHz – 4 MHz) using a designed band pass filter written with Matlab scripts. Then the output was passed to a high-pass filter designed with a cut-off frequency of 50 Hz to remove noise associated with cables and remnant DC signals. The resulting output was passed to a high-pass filter with a cut-off frequency of 200 kHz, to remove the fundamental signal associated with the driving frequency (*f*0). And finally, the output was passed to a comb filter designed to suppress the harmonics (n*f*0). The root-mean square of the resultant acoustic signal was obtained to represent the acoustic strength (time-average shock wave content) of the cavitation. It should be noted that the mean from three data sets were used for all the Vrms values reported in this manuscript.



***Supplemental Fig. S1:*** *Griess test calibration curve obtained from the plot of the mean absorbance obtained at 540 nm from three repeats using standard solutions of NaNO2.*



***Supplemental Fig. S2:*** *Calibration curve obtained from the plot of the mean absorbance obtained at 220 nm from three repeats using standard solutions of KNO3. The standard solution was prepared using KNO₃ purchased from ThermoFisher (99.994% purity, CAS No: 7757-79-1). The KNO₃ was dried in an electric oven at 120 °C for 24 hours prior to being used to make the stock solutions.*



***Supplemental Fig. S3:*** *Calibration curve obtained from the plot of the mean absorbance obtained at 350 nm from three repeats using standard solutions of H2O2. The absorbance at 350 nm was plotted against standard solution concentration (0 – 30 μM, prepared from a 30% w/w stock solution of hydrogen peroxide in water). 600 μL of each standard solution was combined with 3 mL of potassium iodide solution (0.1 M) and 60 μL of ammonium molybdate solution (0.01 M) in a quartz cuvette. After five minutes the mixture was analysed by UV-vis (Agilent Cary 60 UV-vis spectrometer), and the absorbance at 350 nm was recorded.*



***Supplemental Fig. S4:*** *Calibration curve obtained from the plot of the mean absorbance obtained at 350 nm from three repeats using standard solutions of KI. The absorbance at 350 nm was plotted against the standard solution concentration (0 – 50 μM, prepared from KI).*

**Supplemental Table S1:** A summary of reports that have employed the pulse width modulation approach to enhance chemical reactions other than nitrogen fixation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Author(s)** | **Transducer types/frequency used** | **Excitation mode** | **Sample treated** | **Variable used** | **Findings** |
| Sun *et al*,S12 | Ultrasonic horn (planar)/20 kHz | Continuous and pulse width modulation methods | Aqueous solution of potassium iodide (to produce triiodide) | Duty cycle, *t*on and *t*off | The performance of pulse width modulation methods depends on the duty cycle, *t*on. |
| Gutiérrez *et al,S14* | Planar/ 152 kHz | Continuous and pulse width modulation methods | Aerated water (to produce NOx) | Power density, duty cycle, *t*on. | The performance of pulse width modulation was found to be the same with that from continuous method at low power density (1.3 W/cm2) |
| Tuziuti *et al*,S15 | Planar/ 152 kHz | Continuous and pulse width modulation methods | Aqueous solution of potassium iodide (to produce triiodide) | Duty cycle, *t*on and *t*off | The performance of pulse width modulation was found to be 5 times more than that from continuous method. |
| Casadonte *et al*,S16 | Planar/ 400 kHz | Continuous and pulse width modulation methods | Aqueous solution of potassium iodide (to produce triiodide) | Duty cycle | Claimed that for the performance of pulse width modulation to be greater than continuous method only when the peak power is modulated. |
| Xu *et al*,S17 | Planar/ 490 kHz | Continuous and pulse width modulation methods | Wastewater and Aqueous solution of potassium iodide (to produce triiodide) | *t*on and *t*off | Up to 15% performance improvement was achieved at *t*on = *t*off = 0.24 s. |
| Patience *et al*,S18 | Ultrasonic horn (planar) | Continuous and pulse width modulation methods | Pectin extracted | Duty cycle, *t*on and *t*off | At 50% duty cycle, pulse width modulation consumed less than half of the total energy consumed with continuous excitation. |

Most authors have attributed the poor performance of the continuous excitation method to the presence of ‘degassed bubbles’ (referred herein as non-collapsing bubbles) which attenuate the sound wave and limit the reaction zone.S9,S7,S8 Where there is improvement in the performance as a result of using pulse width modulation, the authors typically ascribe this to the presence of ‘residual sound pressure’ and the removal of the ‘degassed bubbles’ during pulse interval. S7,S10

The source of oxygen during ultrasonic formation of NOx− was investigated. 20 mL of both degassed and non-degassed deionised water were sonicated each for 2 minutes with both continuous wave and pulse width modulation excitation and the samples were then analysed for NOx− and H2O2. The concentration of H2O2 produced from degassed water was small as compared to that obtained with aerated samples (see Supplementary Table 2 below). The analysed samples also showed only a negligible presence of NOx−, see Table 4 in the main manuscript. Both of these observations were attributed to a relative lack of dissolved oxygen (and nitrogen) in the case of the degassed samples.

**Supplemental Table S2:** The amount of H2O2 obtained from sonication of water samples. Degassed samples of deionised water were produced by purging 20 mL of deionised water with Ar for 30 minutes. The concentration of H2O2 was quantified using the calibration curve in Supplementary Fig 3.

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| --- | --- | --- | --- |
| **2 minutes sonication conditions**   |  |  | | --- | --- | |  |  | | **[H2O2] (µM)** |
| Degassed water (continuous wave) | 4.64 ± 0.54 |
| Degassed water (pulse width modulation) | 4.72 ± 0.37 |
| Deionised water sparging with air (continuous wave) | 23.59 ± 0.59 |
| Deionised water sparging with air (pulse width modulation) | 31.54 ± 0.79 |

Sonicating degassed and non-degassed KI solutions is known to scavenge hydroxyl radicals, resulting in the formation of triiodide due to the oxidation of KI by hydroxyl radicals, as per:

•OH + I−  → OH− + I

I + I−  → I2−

2I2− → I2 + 2I–

I2 + I− → I3−

Under both degassed and non-degassed conditions, the concentrations of triiodide were obtained and tabulated as shown in Supplementary Table 3 below. The concentration of triiodide from non-degassed samples was found to be approximately double that which was obtained from solutions that had been degassed. This suggests that a primary mechanism for hydroxyl radical generation is via the splitting of water, as shown in Equations 4 and 5 of the main manuscript. The proposed mechanism for the ultrasonic nitrogen fixation process is summarised in Fig. 6 of the main manuscript.

**Supplemental Table S3:** Triiodide formation as a result of oxidation of KI by hydroxyl radicals. 20 mL samples of 0.1 M of KI were sonicated for 2 minutes in the prototype sonoreactor with its lid closed under air-sparged and unsparged conditions. For the degassed samples, the solutions were first purged with Ar for 30 minutes, and then the solutions were sonicated for 2 minutes. The triiodide concentration was quantified using the calibration curve in Supplementary Fig 4.

|  |  |
| --- | --- |
| **Experimental Conditions** | **Triiodide concentration [I3−] (µM)** |
| 2 minutes of ultrasound irradiation of 0.1 M of KI with lid sealed (continuous wave) | 20.38 ± 0.01 |
| 2 minutes of ultrasound irradiation of 0.1 M KI with lid sealed, degassed with Ar (continuous wave) | 9.18 ± 0.01 |
| 2 minutes of ultrasound irradiation of 0.1 M of KI with lid sealed (pulse width modulation) | 28.80 ± 0.03 |
| 2 minutes of ultrasound irradiation of 0.1 M of KI with lid sealed, degassed with Ar (pulse width modulation) | 14.50 ± 0.04 |



***Supplemental Fig. S5:*** *The effect of pulse duration on both time-average shock wave content and the NOx−* *concentrations at the optimum pulse interval of 80 ms. Each data point represents the mean from three sets of data obtained under the same conditions, with error bars corresponding to the standard deviation from the mean for each point.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Supplementary Fig. 5. illustrates how both Vrms and NOx− concentration vary with pulse duration at the optimal pulse interval of 80 ms. Pulse duration appears to be inversely proportional to Vrms. However, the relationship between NOx− concentration and pulse duration is very much non-linear. Initially, increasing the pulse duration from 2 to 4 ms results in an increase in NOx− concentration, but subsequently, NOx− concentration decreases as the pulse duration increases from 4 to 7 ms. These observations can be explained as follows. Given that the pulse interval and pulse amplitude remain constant, the number of cavitation bubbles is expected to remain the same as long as the sparged air is maintained at a constant flow rate. At short pulse durations (where Vrms was observed to be highest) a larger number of bursts is required in order to achieve the same overall total irradiation time. Consequently, the cumulative Vrms obtained over these bursts is higher. Conversely, a longer pulse duration results in a lower number of bursts, leading to a lower Vrms. Moreover, when the pulse duration is extended, the sparged gas faces difficulty in escaping the transverse acoustic pressure generated by the focused transducer. Instead, it tends to coalesce, forming large non-collapsing bubbles around the focal point within the reactor. As a result, the proper saturation of the sample with the sparged air is hindered. This leads to the generation of fewer cavitating bubbles and, consequently, lower Vrms, as shown in Supplementary Fig. 6(a) and Supplementary Videos 5 and 6. |  |  |  |  |

For NOx− on the other hand, increasing the pulse duration from 2 to 4 ms resulted in an increase in NOx− concentration as shown in Supplementary Fig. 4. A similar finding was reported in5 where an increase in pulse duration led to higher NOx− concentration; the lower concentration of NOx− at shorter pulse durations was attributed to insufficient time for chemical activation. In our case, when the pulse duration was increased from 4 to 7 ms, the NOx− concentration decreased. This decrease is explained by the formation of large non-collapsing bubbles from coalescence of the sparged air around the transducer focus (see Supplementary Fig. 6(a)), preventing proper saturation of the solution by the sparged air (see Supplementary Video 5). As a result, fewer active cavitation bubbles responsible for nitrogen fixation are generated, as shown in Supplementary Fig. 6(a).

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***Supplemental Fig. S6:*** *High-speed imaging collected at 20,000 fps showing the effect of pulse duration on the number of active cavitation bubbles: (a) overlay of the focal region of the simulated focused ultrasound at pulse duration = 5 ms and pulse interval 80 ms; the region in blue indicates the quiet part (low pressure region) and the yellow region indicates where the pressure field is most intense (the focus); (b) high speed imaging at pulse duration = 4 ms, pulse interval = 80 ms. In the case of frame (a), the formation of large non-collapsing bubbles within the reactor inhibits the proper saturation of the solution with air. The scale bar indicates 5 mm.*

Supplementary Fig. 7 presents the Vrms plot obtained at 200 ms intervals over a duration of 1 second. The steady decline in the Vrms value occurred due to the ongoing depletion of active cavitating bubbles and the attenuation of the sound field caused by the presence of the large non-collapsing bubbles.

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***Supplemental Fig. S7:*** *Time-averaged shock waves content (Vrms) collected at 200 ms time intervals for one second of continuous sonication.*

**Design and construction of the prototype sonoreactor**

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***Supplemental Fig. S8:*** *Detailed drawings of the prototype sonoreactor (working chamber). This was made from steel of thickness 3 mm. The sonoreactor had a diameter of 60 mm and a height of 45 mm.*

The sonoreactor was constructed with an outer jacket equipped with an inlet and outlet for circulating cold water, facilitating temperature control. These features ensured that the reactor could operate for extended periods while maintaining a controlled temperature.

The 40 kHz, 50 W transducer used was bolted and glued to the base of the reactor with the help of an epoxy glue. After construction, the impedance of the device was then measured using an impedance analyzer (Keysight Technologies E4990A). As shown in Supplementary Fig. 8b, the primary resonance of the device was found to occur at approximately 36 kHz which was less than the 40 kHz transducer used. The reduction in the resonance frequency is due to mechanical loading on the transducer. The impedance at this resonance frequency was found to be approximately157 Ω at angle of −6°.



***Supplemental Fig. 9:*** *Impedance measurement of the sonoreactor after complete fabrication. Knowledge of the impedance allowed the selection of an appropriate driver for the device.*

**Estimation of Acoustic Power Density of the Prototype Sonoreactor**

The acoustic power density of the £20prototype sonoreactor was obtained using a calorimetry method.S11,S21 In this method, a thermocouple with a data logger (USB TC-08, Pico Technology) was submerged vertically at a fixed distance (~5 mm) from the liquid surface into 20 mL of deionized water contained in the sonoreactor and the water was then sonicated for 150 s. The thermocouple was held at this position with the help of a steel support clamp stand. Three sets of readings were taken for each operating setting from which the mean ± standard deviation was computed and plotted. Supplementary Fig. 9 shows the rise in temperature with time during sonication as obtained from the thermocouple data logger.



***Supplemental Fig. S10:*** *Temperature rise per second produced by continuous sonication of 20 mL deionized water for 150 s for three separate trials. The water was replaced for each run, which is why each run has a slightly different starting temperature.*

The acoustic power (W) was estimated from the slope of the plots (i.e. the temperature change versus time) using equation (1). The acoustic power density was then calculated by dividing the acoustic power obtained by the volume used.S21

(1)

Where is the specific heat capacity of water (4.185 J g-1), M is the mass of water (g), and is the rise in temperature per second.

**Description of Supplementary Videos**

**Supplemental Video 1:** A movie showing the simulation profile of the typical focused ultrasound used in this research. The simulation was carried out using finite elements with OnScale software. The focused ultrasound transducer was modelled in 3-D based on the available information provided by the manufacturer (Sonic Concepts Inc). Information such as the internal diameter (20 mm-Ø), outside diameter (110 mm-Ø), focal distance of 68 mm, and its radius of curvature of 70 mm were all provided but the active material was not provided. However, we have used PZT4 as the active material in our simulation. The focused ultrasound was modelled surrounded by water elements and simulated using 20 mesh per wavelength to achieve the pressure distribution field shown in Supplementary Video 1. The focal length, focal width, and the focal distance within the pressure distribution were found to be ~ 22.5 mm, ~ 5 mm, and ~ 68 mm, respectively. These values are comparable with those given by the manufacturer which are focal length of 22.36 mm and focal width of 4.87 mms.S13

**Supplemental Video 2:** A movie showing the high-speed frames collected at 1,000,000 fps to capture the oscillation and collapse of the cavitating bubbles, especially at the moment of shockwave generation.

**Supplemental Video 3:** A movie showing the high-speed frames collected at 20,000 fps of cavitation at a pulse amplitude of 500 mVpp. The cavitation bubbles are seen in clusters due to the attraction force between them (Bjerknes forces) but their numbers are few because of the lower acoustic pressure (~2.32 ± 0.21 MPa) associated with this amplitude.

**Supplemental Video 4:** A movie showing the high-speed frames collected at 20,000 fps of cavitation at a pulse amplitude of 800 mVpp. The number of clustering cavitation bubbles is observed to increase, in a much larger numbers due to the increase in the excitation voltage (800 mVpp) vis-à-vis the acoustic pressure of ~ 3.91± 0.35 MPa.

**Supplemental Video 5:** A movie showing the high-speed frames collected at 20,000 fps of cavitation at a pulse amplitude of 1000 mVpp. The highest number of cavitation bubbles were observed at this voltage excitation, corresponding to an acoustic pressure of ~ 4.69 ± 0.42 MPa. The cavitation bubbles are seen to be more energetic and oscillate faster and collapse more violently resulting in the highest time-average shock wave content, Vrms observed.

**Supplemental Video 6:** A movie showing the high-speed frames collected at 20,000 fps of big non-collapsing bubbles formed as result of the coalesce of the sparged air during a pulse duration of 5 ms. The observation of a big non-collapsing bubble is due to the coalesce of the sparged air; this bubble hinders proper saturation of the air and hence, the number of nucleation points is greatly reduced.

**Supplemental Table S4:** Different input protocols at a pulse amplitude of 1000 mVpp and 20 s total sonication time (pulse duration × number of bursts) for the setup shown in Fig. 3 in the main text.

|  |  |  |  |
| --- | --- | --- | --- |
| **Protocol** | **Pulse duration [ms]** | **Pulse Interval [ms]** | **Number of bursts** |
| 1 | 2 | 20, 40, 80, 120, and 160 | 10,000 |
| 2 | 4 | 20, 40, 80, 120, and 160 | 5,000 |
| 3 | 5 | 20, 40, 80, 120, and 160 | 4,000 |
| 4 | 7 | 20, 40, 80, 120, and 160 | 2857 |

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