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Ultrasonic transparency of sonication tubes exposed to various frequencies: A metrological evaluation of modifications and uncertainty of acoustic pressures

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ABSTRACT

Correlations between biological phenomena and ultrasonic exposure often involve mechanical and thermal effects. Cavitation proved capable of interacting with other factors, making awkward the evaluation of their individual effects. In microbiological research, the presence of a dual effect of ultrasound on microorganisms, namely bactericidal and stimulating, required development of methods enabling analysis of ultrasonic field effects, shielded from those of cavitation. This work shows how acoustic wave action may be analyzed with a metrological approach, excluding cavitational effect and measuring acoustic pressure acting upon a sonication tube. Results show how such a goal was achieved in a repeatable and reproducible way, avoiding acoustic wave degeneration.

1. Introduction

Ultrasound (US) and ultrasonic techniques have a broad range of applications, covering e.g. mechanical, chemical, electronics, food industry typically for decontamination purposes, not to mention their usefulness in medical diagnostics and therapeutics. In medical applications, characterization of US activity on eukaryotic and prokaryotic still require substantial work. As far as biological effects are concerned, two independent parameters of the ultrasonic wave, intensity and frequency, determine treatment effectiveness; thus surgical effects on soft tissues require high frequency and high intensity levels, while stimulation of cellular metabolism calls for low frequency and low intensity [1]. Some regions of this two-parameters space were explored and related effects reported in literature, particularly in connection with prokaryotic cells metabolism. Thermosonic, manosonic and manothermosonic US treatments are mentioned by some authors for their antibiofilm action, while diagnostic ultrasounds are sometimes described as enhancers of bacterial viability [1]. These ambiguous effects caused some misunderstandings about the influence of ultrasounds on prokaryotic cells. In the 2003 the works of Pitt and Piyasena showed clearly that, when bacteria are exposed to an ultrasonic field, both phenomena of destruction and stimulation may coexist and interfere [1–3]. This competition can have different outcomes owing to various influencing factors, such as bacterial species involved, nature of medium through which ultrasonic waves propagate, presence of cavitation

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phenomena and, last but not least, structure of bacterial community (planktonic or biofilm form) [1,4–8]. Cavitation ranks high among the most studied influencing factors [3,9–11]; it occurs when high intensity ultrasonic waves, with frequencies typically between 10 kHz and 1 MHz, originate negative pressure in a liquid medium [1]. This leads to formation of gas and vapor bubbles which eventually collapse, generating shock waves and high temperature spots [12,13]. Cavitation bubbles typically target permeability of cellular membrane, with a strong bactericidal effect [10,11]. Action of acoustic pressure (p_{AC}) on living cells in absence of cavitation bubbles apparently attracted a minor amount of investigation [2,14]. Furthermore, p_{AC} seems to be associated to an increased cellular oxygenation and nutrient adsorption (favouring the growth rate) [2,15] but also to a greater antibiotic sensibility [16,17]. To our knowledge, precious few data are present in literature about antimicrobial effects of p_{AC} in non-cavitated media. The present work deals with a method aimed at assessing the influence of low intensity ultrasound on the metabolism of prokaryotic cells, *in vitro* planktonic and free floating forms being considered.

2. Experimental apparatus and methods

The effects of low-intensity US on micro-organisms was tested using a modified sonication bath (Branson 3200 Ultrasonic Cleaner, $30 \times 15 \times 15$ cm^3). Built-in electronics of a commercial cleaning tank was replaced by an external broad-band amplifier driven by function generator. To avoid contaminations of culture media from the medium propagating ultrasonic waves, de-mineralized water in our case, prokaryotic cells were grown in test tubes, immersed in the sonication bath [15].

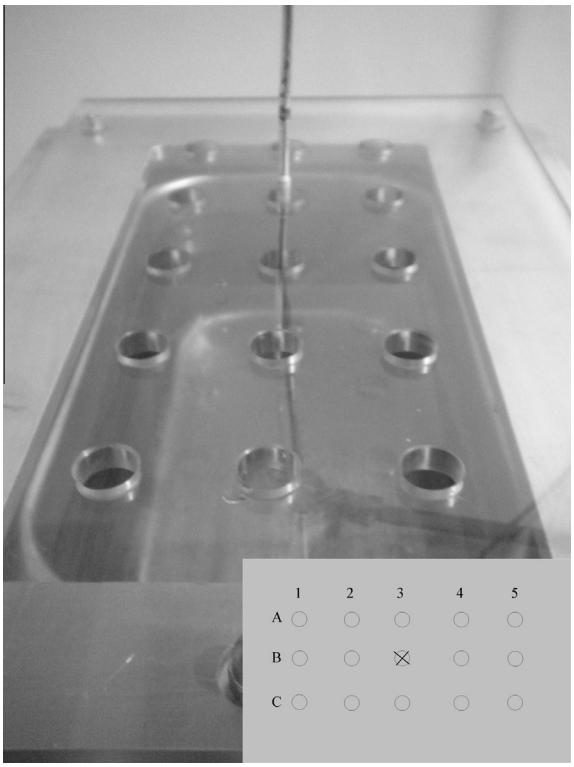

Fig. 1. Sonication bath with the custom positioning system. All the described experiments were performed in the central position 3B.

Exposition of culture medium to ultrasound is affected by position in the bath of test tubes, by their shape and their material. A standard measurement procedure was developed, aimed at measurement reproducibility with

Table 1
Hydrophone calibration. Mean values m and lower limit L_L and upper limit U_L of 95% confidence intervals (uncertainty intervals of sensitivity S), expressed as dB re 1 V/μPa and as μV/Pa, at explored frequencies.

Frequency/kHz	S_{dB}/(dB re 1 V/μPa)			S/(μV/Pa)		
	m	L_L	U_L	m	L_L	U_L
20	−270.6	−272.6	−268.6	$2.96 \cdot 10^{-2}$	$2.35 \cdot 10^{-2}$	$3.73 \cdot 10^{-2}$
21	−272.6	−277.9	−267.4	$2.33 \cdot 10^{-2}$	$1.28 \cdot 10^{-2}$	$4.26 \cdot 10^{-2}$
22	−274.4	−278.2	−270.6	$1.90 \cdot 10^{-2}$	$1.23 \cdot 10^{-2}$	$2.95 \cdot 10^{-2}$
23	−272.6	−274.9	−270.4	$2.34 \cdot 10^{-2}$	$1.80 \cdot 10^{-2}$	$3.03 \cdot 10^{-2}$
24	−268.6	−270.3	−266.8	$3.73 \cdot 10^{-2}$	$3.06 \cdot 10^{-2}$	$4.55 \cdot 10^{-2}$
25	−269.5	−275.8	−263.3	$3.34 \cdot 10^{-2}$	$1.63 \cdot 10^{-2}$	$6.83 \cdot 10^{-2}$
26	−269.6	−271.8	−267.3	$3.32 \cdot 10^{-2}$	$2.56 \cdot 10^{-2}$	$4.31 \cdot 10^{-2}$
27	−266.8	−269.3	−264.3	$4.55 \cdot 10^{-2}$	$3.41 \cdot 10^{-2}$	$6.07 \cdot 10^{-2}$
28	−265.2	−266.0	−264.4	$5.51 \cdot 10^{-2}$	$5.03 \cdot 10^{-2}$	$6.04 \cdot 10^{-2}$
29	−267.3	−273.1	−261.4	$4.33 \cdot 10^{-2}$	$2.20 \cdot 10^{-2}$	$8.50 \cdot 10^{-2}$
30	−267.5	−271.3	−263.7	$4.22 \cdot 10^{-2}$	$2.71 \cdot 10^{-2}$	$6.57 \cdot 10^{-2}$
31	−267.2	−267.8	−266.5	$4.38 \cdot 10^{-2}$	$4.06 \cdot 10^{-2}$	$4.72 \cdot 10^{-2}$
32	−265.5	−266.4	−264.6	$5.32 \cdot 10^{-2}$	$4.79 \cdot 10^{-2}$	$5.89 \cdot 10^{-2}$
33	−263.9	−264.5	−263.4	$6.36 \cdot 10^{-2}$	$5.96 \cdot 10^{-2}$	$6.79 \cdot 10^{-2}$
34	−264.0	−264.8	−263.3	$6.29 \cdot 10^{-2}$	$5.76 \cdot 10^{-2}$	$6.88 \cdot 10^{-2}$
35	−265.0	−266.6	−263.3	$5.63 \cdot 10^{-2}$	$4.65 \cdot 10^{-2}$	$6.82 \cdot 10^{-2}$
36	−264.6	−266.3	−262.8	$5.91 \cdot 10^{-2}$	$4.85 \cdot 10^{-2}$	$7.21 \cdot 10^{-2}$
37	−263.2	−265.5	−260.9	$6.94 \cdot 10^{-2}$	$5.34 \cdot 10^{-2}$	$9.01 \cdot 10^{-2}$
38	−263.3	−264.6	−262.0	$6.86 \cdot 10^{-2}$	$5.90 \cdot 10^{-2}$	$7.98 \cdot 10^{-2}$
39	−264.3	−265.8	−262.8	$6.10 \cdot 10^{-2}$	$5.15 \cdot 10^{-2}$	$7.22 \cdot 10^{-2}$
40	−262.3	−263.0	−261.6	$7.67 \cdot 10^{-2}$	$7.09 \cdot 10^{-2}$	$8.30 \cdot 10^{-2}$