BEHAVIOUR OF SUSPENDED PARTICLES IN WATER-RICH ETHANOL MIXTURES IRRADIATED WITH AN ULTRASONIC STANDING WAVE

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Abstract

Ultrasonic resonators are successfully used for the separation of suspended particles or cells, e.g. as cells filters in biotechnology [1,2]. These systems are based on the radiation forces spatially arranging the particles in the pressure nodal planes of the quasi-standing ultrasonic wave field.
Suspensions of yeast in water-rich ethanol mixtures showed an unexpected breakdown of the spatial distribution above certain concentrations of ethanol, the cells were turbulently mixed in the separation system.
This effect was explained to some extend by the non-linear behaviour of the speed of sound of such mixtures over rising ethanol concentration and the resulting decrease of the acoustic contrast factor of the suspension. However, this explanation turned out to be not fully exhaustive as turbulences as well occurred with latex beads. The material properties of these beads should have suppressed the turbulences when used as the solid phase in water-rich ethanol mixtures.

Introduction

Ultrasonic separation technology [1] nowadays is at a stage where applications of practical importance become visible. The main advantages of cell filters based on this technique are the complete absence of moving parts and therefore no filter cakes or filter fouling. The systems can be hot-steam sterilised in-situ, the used materials such as stainless steel and glass are bio-compatible. Furthermore, an extensive literature on the theory of the interaction of ultrasonic waves with particles exists, and highly advanced piezoelectric transducers and driving electronics are available.
Ultrasonically Enhanced Settling (UES) is one utilisation of particle manipulation by ultrasound waves which has been developed during the last decade up to successful applications in industrial environments. The principle here is it to locally increase the particle concentration by a standing ultrasonic field, which results in loose aggregates stabilised by the ultrasound within certain regions of the sound field. These aggregates settle at the bottom of the vessel due to their decreased friction according to Stokes' law. This subsequently delivers an increase of sediment per time. Thus the build up of aggregates by *ultrasound enhances* the *settling.*
The application of UES is especially successful as cell filters in biotechnology [2]. Recently, a substantial study about the applicability of commercially available UES systems in brewing technology was triggered. It was shown that more than 99.5% of suspended yeast cells could be retained [3], no influence of ultrasound on the viability of the yeast cells was detected [4].
The most important process factor in the brewing regime is ethanol (EtOH), the end-product of the fermentation. Surprisingly the presence of EtOH did alter the behaviour of a yeast suspension in the separation system completely. The cells did not form planes in the sound pressure nodes as expected, but were turbulently driven through the filter cavity. This led to the breakdown of the particle ordering and consequently the separation efficiency of the acoustic filter was severely impaired.
No explanation was at hand for this behaviour at first. During the investigations it was shown, that the ultrasonic quasi-standing wave field was not significantly altered by the presence of EtOH. Furthermore, a chemical assay showed no evidence of inertial cavitation [5], which was suspected to represent an additional source of forces exerted on the particles and thus to disturb the high regularity of the spatial distribution brought about by the ultrasound. This result was also important in respect to an earlier report of the turbulence to go along with an impaired viability of yeast cells sonicated in a 12%($^{v}/_{v}$) ethanol-water mixture [4].
The scope of the presented paper was it therefore to further investigate the question why ultrasonic separation of yeast cells does not take place in water-rich EtOH mixtures. Furthermore some assumptions that will be presented were supposed to be tested by the use of other particles than yeast cells.

Methods

Experimental Set-up and Separation Device
The industrial UES system (USSD-05, Anton Paar GmbH, Graz, Austria) was used for the experiments with yeast cells in batch set-up. The electrical signal was delivered by an amplifier (USCS-05, Anton Paar GmbH, Austria) equipped with an automatic

frequency control (AFC). The ultrasound was emitted by a PZT-glass compound transducer in horizontal direction towards a glass reflector. Between the transducer and the reflector two compartments divided by an acoustically transparent vertical foil were located: a cooling volume with water circulation through it to avoid the transducer to heat the suspension and the active volume filled with the suspension.
Experiments with latex beads were conducted with a small experimental resonator (4 mL) comprising two transducers facing shown in Figure 1.

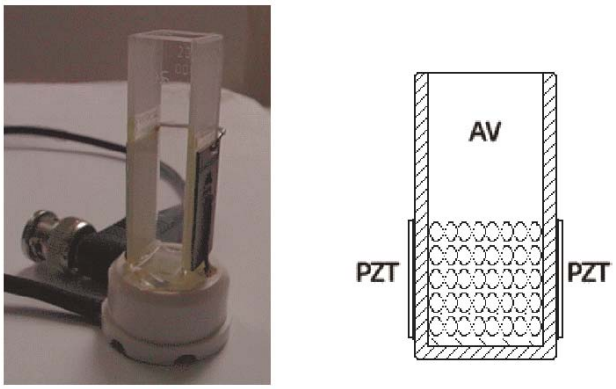

Figure 1 : Experimental resonator with two transducers used for experiments with suspensions of latex beads. Both PZT ceramics were driven by the same frequency source FPS2540. The active volume (AV) was holding the suspension.

The device was driven by a Frequency Power Synthesizer (FPS 2540, PSI, Austria).

Cultivation and handling of suspended Saccharomyces cerevisiae
One colony was retrieved from a plate with a loop and seeded in malt extract broth (0.4 g in 40 mL H_2O). This inoculate was left overnight in a 30°C incubator provided with a orbital shaker table (150 rpm). Subsequently, 5 mL of this culture were added to 95 mL of fresh malt extract broth (2 g in 100 mL H_2O) and let grow for 24 hours in the same incubator.
The culture was centrifuged at 3800 rpm in a Sorvall centrifuge for 10 minutes and the precipitate was re-suspended in 100 mL saline (0.9 g NaCl in H_2O) or water. An appropriate volume of 99% EtOH was added to the sample suspension to reach the respective alcohol concentration, when required.

In-situ Measurement of the Electrical Admittance Spectrum
For the admittance measurements at true electrical power input settings in the working range of the UES system a different device, the Frequency Power Synthesizer FPS 2540 (Sonosep Technologies, Canada) was used. It was controlled by a computer program delivering the drive voltage and current amplitudes and the electric phase angle.

Preparation of suspension with latex beads
Polystyrene latex beads (Bangs Laboratories) with a particle size distribution similar to yeast cells (3-10 µm) were used as solid phase. The host liquid was a mixture of water and 99% EtOH. Both liquids were degassed by boiling prior to the preparation of the mixture. This mixtures with a content of 1% to 4% EtOH were additionally degassed for 10 minutes at an under pressure of –80 kPa.
The behaviour of the suspension during the first three minutes of sonication (2.2 MHz, 2 W) was closely observed and described.

Results

As the mere presence of EtOH was assumed to be the reason for the turbulences an attempt was made to examine the concentration at which the unusual behaviour sets in. Suspensions of yeast were used in the separation system, the EtOH concentration was increased in steps until the separation efficiency showed a significant decrease indicating the breakdown of the spatial order.
Figure 2 shows the results of trials with yeast cells in water (squares) where turbulence was not observed until the concentration of EtOH reached 8-9%($^{v}/_{v}$). When physiological saline was used as host liquid (circles) an influence of EtOH was not detected until the concentration had reached 15%($^{v}/_{v}$).
As the EtOH content was increased in steps of 1%($^{v}/_{v}$), it could be established that the lack of spatial ordering is a sudden process “hitting” in rather than gradually decreasing the separation efficiency. Moreover the trial with a yeast water suspension (squares) in Figure 2 showed that one was dealing with a reversible process. The breakdown in this experiment was observed first at 9%($^{v}/_{v}$). Subsequently the EtOH concentration was decreased again to 8%($^{v}/_{v}$) by adding water. At this concentration the proper arrangement of the cells was re-established.
The observed phenomenon of turbulence was similar to the effect of the acoustic radiation pressure in a progressive wave. In literature the terms “Eckhardt streaming” or “quartz-wind” are found. To test this hypothesis the true electrical power input spectrum of the separation system with fillings of a suspension of

yeast cells present in an 8%($^{v}/_{v}$) EtOH-water mixture was recorded at frequencies between 1.9 MHz and 2.3 MHz at constant drive voltage levels.

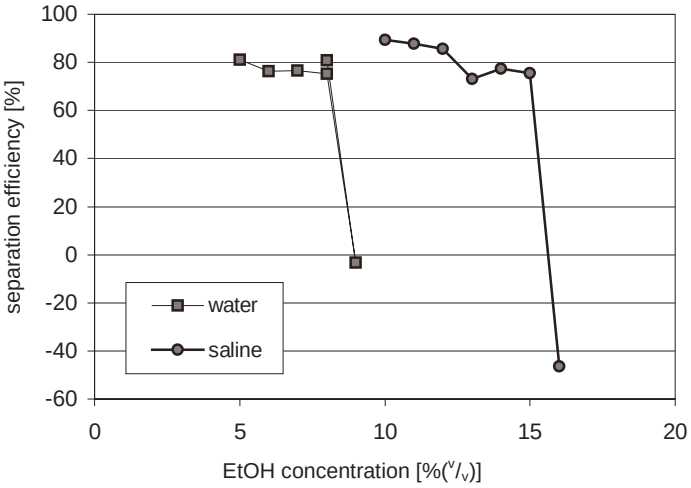

Figure 2 : Evaluation of the EtOH concentration at which the spatial arrangement of yeast cells breaks down. Suspensions of yeast cells in water (squares) and in 0.9%($^{w}/_{v}$) NaCl (circles) were used.

The measured power spectra showed a significant increase of the resonance peak widths on higher settings of the impressed drive voltage level. In Figure 3 the result is shown for voltage settings of 10 V, 18 V and 28 V respectively. Clearly the spectrum did loose structure caused by the overlapping of the peaks at higher levels of true electrical power input. This behaviour was typical for a decrease of the resonance quality factor which in turn was an indication for an energy consuming process. In correspondence with the turbulent movement of the suspended cells observed in the resonator chamber, the reason of the increased energy loss of the acoustic field was easily identified: part of the acoustic energy was transformed into the kinetic energy of the moving particles. In consistence with these observations Figure 3 suggests, that the velocity of the cells turbulently driven through the separation system increased with an increase of electrical input power, resonance peaks were wider for higher settings.

As in an earlier study [5] no changes of the standing-progressive wave ratio due to the addition of EtOH were detected. Therefore it was concluded that the *interaction* between the ultrasonic field and the particles might have changed. More precisely the force exerted on the particles was supposed to be different in water-rich EtOH mixtures from the respective force in pure water.

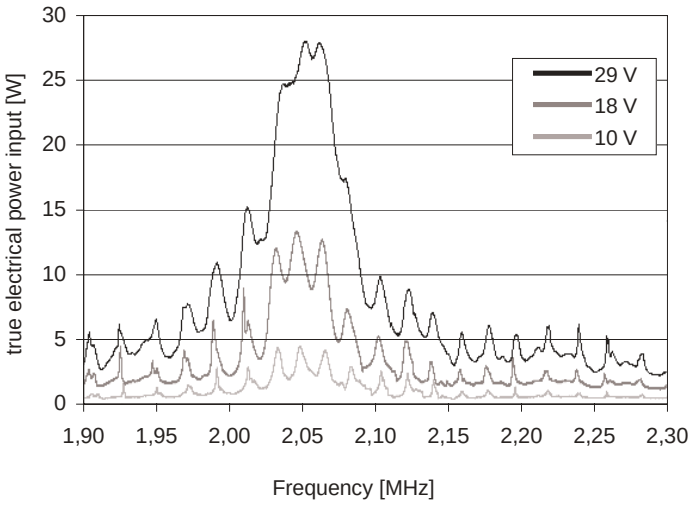

Figure 3 : Power spectra of the separation system filled with yeast (approximately $5 \cdot 10^6$ cells/mL) suspended in 8%($^{v}/_{v}$) water-ETOH at impressed (load independent) drive voltage settings of 10 V, 18 V and 29 V. A decrease of the resonance quality factor with increasing true electric power input was detected.

The ratio of the time-averaged axial primary radiation forces of a progressive wave F_p and a standing wave F_s is given in Eq. (1), the expressions were published in [6]. They are valid for a compressible sphere in a host fluid of the mass density ρ. The other symbols in Eq. (1) are the particle's radius *a* and the wave number *k*. The amplitudes of the progressive and standing wave field's velocity potential are denoted by $\hat{\Phi}_p$ and $\hat{\Phi}_s$, respectively. The coefficients $K_p(\lambda,\sigma)$ and $K_s(\lambda,\sigma)$ are called acoustic contrast factor of the progressive and standing wave respectively. They are dependent on the density ratio $\lambda = \rho_0/\rho$ of particle and liquid and the speed of sound ratio $\sigma = \upsilon_0/\upsilon$, whereby the subscript 0 denotes the respective value for the particle.

$$\frac{\left\langle F_p\left(K_p\right)\right\rangle}{\left\langle F_s\left(x,K_s\right)\right\rangle} = \frac{2\pi\rho\cdot\hat{\Phi}_p^2\left(ka\right)^6 K_p\left(\lambda,\sigma\right)}{4\pi\rho\cdot\hat{\Phi}_s^2\left(ka\right)^3 K_s\left(\lambda,\sigma\right)\sin\left(2kx\right)} = \tag{1}$$

$$= \underbrace{\left(ka\right)^3}_{\text{I}} \cdot \underbrace{\frac{K_p\left(\lambda,\sigma\right)}{K_s\left(\lambda,\sigma\right)}}_{\text{II}} \cdot \underbrace{\frac{\hat{\Phi}_p^2}{2\hat{\Phi}_s^2}}_{\text{III}} \cdot \underbrace{\frac{1}{\sin\left(2kx\right)}}_{\text{VI}} \quad .$$

Eq. (1) delivers four factors expressing the dependencies of the radiation forces on various properties of the suspension:

I. describes the influence of the frequency and the particle size; due to the limiting condition $ka \ll 1$ the radiation force of a standing wave in general is much stronger than that of a progressive wave;
II. represents the influence of the speed of sound and the mass density of the particle and the host liquid;
III. describes the amplitude ratio of the progressive and the standing wave and thus is dependent on the effective attenuation, i.e. the viscous damping of the suspension and additional losses as well as the reflection coefficient of the reflector terminating the resonator chamber;
IV. describes the periodic structure of the standing wave's envelope;

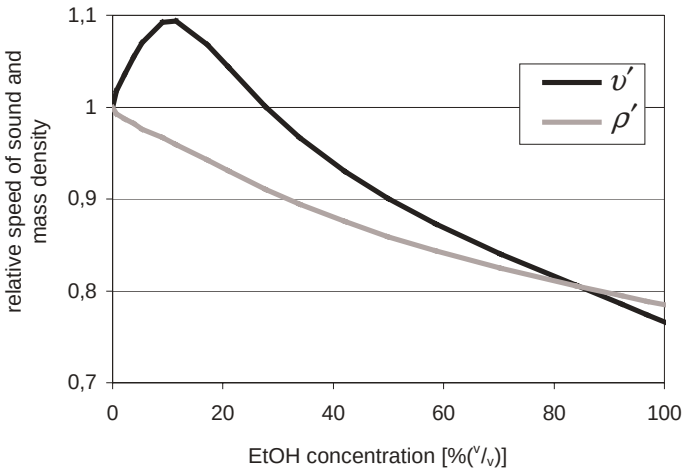

Figure 4 : Relative speed of sound (υ') and mass density (ρ') of water-EtOH mixtures at 25°C in comparison to water (data published by Lara and Desnoyers [7]).

As nothing else but the material properties of the host liquid were changed due to the addition of EtOH, the speed of sound and mass density were investigated (see Figure 4). A superficial examination of water-EtOH mixtures in comparison to the respective material properties of pure water [7,8] delivered a deviation of some 10% for the speed of sound and less than that for the mass density at 12%(v/v) EtOH. At first glance, this did not seem to be an effect strong enough to cause such fundamental changes in behaviour.

A closer look however revealed the precise circumstances to be different. The material properties of the host liquid alone do not yield the whole picture, one has to take the dispersed particle into account as well.

$$K_p(\lambda,\sigma) = \frac{1}{(1+2\lambda)^2}\left[\left(\lambda - \frac{1+2\lambda}{3\lambda\sigma^2}\right)^2 + \frac{2}{9}(1-\lambda)^2\right] \qquad (2)$$

$$K_s(\lambda,\sigma) = \frac{1}{3}\left(\frac{5\lambda-2}{2\lambda+1} - \frac{1}{\lambda\sigma^2}\right) \quad .$$

The ruling coefficient is the acoustic contrast factor ratio, as of term II of Eq. (1). It turned out that the acoustic contrast factors of the progressive and the standing wave as given in Eq. (2), respectively, do not both vanish at the same values of λ and σ.

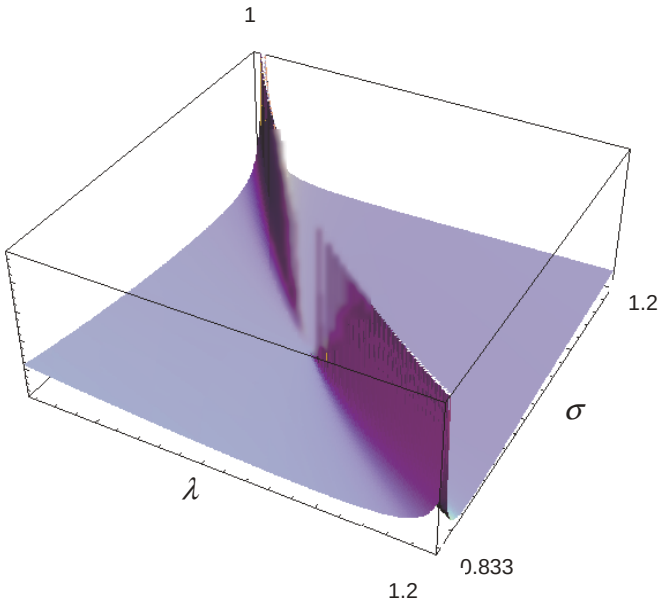

Figure 5 : Surface of the ratio of the acoustic contrast factors of a progressive and standing wave as of term II in Eq. (1). Ratio values above one are not shown. A singularity, i.e. a region where the acoustic contrast factor of the standing wave becomes zero but not the acoustic contrast factor of the progressive wave is clearly indicated by high values of the ratio.

Thus one deals with a *singularity* which lets the radiation force contribution of *any* progressive wave present in the resonator exceed that of the standing wave for certain combinations of the mentioned material properties of particles and host liquid. A progressive wave however can be assumed to be present in any realistic separation system, it is even necessary to transport the energy that compensates for

the losses within the resonator (a standing wave does not transport energy).
Figure 5 shows the ratio of the acoustic contrast factor of the propagating wave over the acoustic contrast factor of the standing wave $K_p(\lambda,\sigma)/K_s(\lambda,\sigma)$ calculated from term II. The region where the acoustic contrast factor of the standing wave becomes small and vanishes are clearly indicated by high values of the ratio. The acoustic contrast factor ratio was calculated from the explicit expressions in Eq. (2). The resulting values for λ and σ for yeast suspended in water-EtOH mixtures are assigned to the axes of Figure 5.

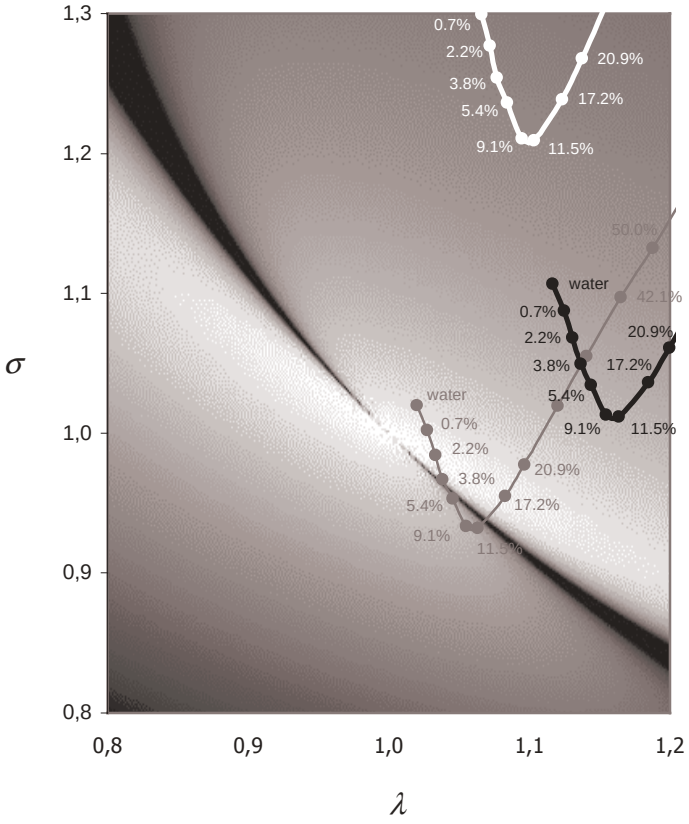

Figure 6 : Acoustic contrast factor ratio as of Eq. (1) of yeast cells (black), modified yeast cells (grey) and latex beads (white) suspended in water-EtOH mixtures. Numbers represent the EtOH concentration.

Figure 6 shows the acoustic contrast factor ratio from above, each point on the plane of Figure 6 represent a suspension in terms of specific values of the mass density and speed of sound ratio (λ,σ) of the suspended particles and the host liquid. In this illustration the acoustic contrast factor ratio $K_p(\lambda,\sigma)/K_s(\lambda,\sigma)$ is represented by the shading of the (λ,σ) plane, darker regions represent higher values as of term II of Eq. (1). The evaluation of the acoustic contrast ratio for yeast cells suspended in water-EtOH mixtures was conducted with material properties for the water-EtOH mixtures as shown in Figure 4. For the black line in Figure 4 the mass density of yeast ρ_{yeast} = 1114 kg m^{-3} was used [9], the speed of sound value was estimated from the measured compressibility of erythrocytes [10] to υ_{yeast} = 1642 m s^{-1}. With increasing EtOH concentration the "path" approaches the area where the acoustic contrast factor of the standing wave vanishes, however, critical regions are not reached.
As the particles here were yeast cells one may assume changes of their "material properties". The relaxing influence of EtOH on the cell envelope presumably could decrease the speed of sound within the cell. Furthermore, mass transfer through the cells' wall/membrane occurs, hence the mass density might change too [11]. Therefore the calculation was repeated with modified values of ρ_{yeast} = 1018 kg m^{-3} and υ_{yeast} = 1512 m s^{-1}, hence $\lambda=\sigma$ =1.02 when suspended in water. The grey line in Figure 6 shows the acoustic contrast factor ratio $K_p(\lambda,\sigma)/K_s(\lambda,\sigma)$ for suspensions of such particles in water-EtOH mixtures. At EtOH concentrations around 10%($^{v}/_{v}$) the contrast ratio gets very close to those regions where the acoustic contrast factor of the standing wave and hence the cell-ordering effect would vanish.
To further confirm the explanation a similar experiment was conducted with a different particle type: Latex beads of the same particle size range like yeast cells suspended in water-rich EtOH mixtures were used. Sonication was performed in a smaller resonator with two transducers facing each other and therefore lower gradients of the sound field. The result is listed in Table 1. Clearly the spatial distribution was instable for very low concentrations of EtOH.

Table 1 : Behaviour of latex beads suspended in water-rich EtOH mixtures sonicated at 2.2 MHz

EtOH conc.	Observations
1%	slow turbulences occurred after 2-40 s
3%	turbulences occurred after 1-25 s
4%	turbulent from the beginning

The polystyrene latex beads had a mass density of ρ_{latex} = 1056 kg m^{-3} and a speed of sound of υ_{latex} = 1962 m s^{-1}. This leads to a mass density ratio relative to water of λ=1.06 and a speed of sound ratio of σ =1.32. The development of the acoustic contrast ratio $K_p(\lambda,\sigma)/K_s(\lambda,\sigma)$ over rising EtOH

concentration for this latex particle is shown as white line in Figure 6. Over the EtOH concentration used in the described experiment the acoustic contrast ratio is far off the singularity!

Conclusion

The presented experiments lead to the following conclusions:

- The breakdown of the spatial ordering by the ultrasonic field of yeast cells occurred suddenly at a certain EtOH concentration and was reversible, i.e. the addition of water did terminate the streaming and re-established the ultrasonically induced spatial order.
- A quasi-standing wave was still present when yeast cells were suspended in a 8%($^{v}/_{v}$) EtOH-water mixture although turbulence was observed in the separation system. However, an additional energy consumption was detected by a decrease of the resonance quality factor at higher settings of input power. This was interpreted due to the drain of kinetic energy by the moving particles within the resonators' active volume.
- The material properties speed of sound and mass density of water-rich EtOH mixtures were found to be possibly responsible for the breakdown of yeast observed. The influences on the acoustic contrast factors between the yeast cells and the host liquid led to acoustic contrast factor ratios which yielded a contribution of the primary radiation force of the standing wave which can be smaller than that of the progressive wave. This became most evident when certain changes of the respective properties of yeast cells were assumed.
- However this explanation could not be used for the observed turbulences of latex particles and water-rich EtOH mixtures. The material properties mass density and speed of sound of polystyrene latex lead to a development of the acoustic contrast factor ratio which was far off the mentioned singularity.

Discussion

The presented calculations of the acoustic contrast factor ratio, especially with hypothetical yeast cells with modified speed of sound and mass density, shed some light on the unexpected behaviour of turbulence in water-rich EtOH mixtures when subjected to a standing acoustic field.

However this explanation was not exhaustive and could not be used to explain the behaviour of latex beads in water-rich EtOH mixtures. If not other factors than the primary radiation forces are responsible for the observed turbulences other terms of the comparison of the forces exerted on the particles by the progressive and the standing wave as of Eq. (1) have to be taken into account.

The experiment in Figure 3 suggested that damping or dissipative processes might be of relevance. Such energy consuming mechanisms are represented for arbitrary amplitudes of the progressive and the standing wave by term III in Eq. (1). For further investigation these amplitudes have to be brought into connection with a quasi-standing wave, i.e. a standing wave superposed with a progressive wave described by a velocity potential $\tilde{\Phi}_{qs}$ as given by Eq. (3),

$$\tilde{\Phi}_{qs} = \hat{\Phi} \cdot e^{i(\omega t - kx)} + \hat{\Phi}' \cdot e^{i(\omega t + kx)} \quad \text{with} \quad \hat{\Phi}' < \hat{\Phi} \tag{3}$$

In Eq. (3) ω is the angular frequency, t the time, k the wave number and $\hat{\Phi}$ and $\hat{\Phi}'$ are the amplitudes of the waves traveling in positive and negative x-direction through the resonator respectively. The decrease of the amplitude of the reflected wave $\hat{\Phi}'$ can be explained by the consideration of e.g.

$$\text{damping:} \quad \hat{\Phi} \to \hat{\Phi} \cdot e^{-\alpha x} \quad , \quad \hat{\Phi}' \to \hat{\Phi}' \cdot e^{-\alpha(2l - x)} \tag{4}$$

with an attenuation coefficient α or by incomplete

$$\text{reflection:} \quad \hat{\Phi}' \to R \cdot \hat{\Phi} \tag{5}$$

with a reflection coefficient R<1. The time-averaged primary radiation force exerted on a particle by a quasi-standing wave was calculated by Hasegawa [12] to be

$$\left\langle F_{qs}(x) \right\rangle = \left[1 - \left(\frac{\hat{\Phi}'}{\hat{\Phi}} \right)^2 \right] \left\langle F_p\left(K_p\right) \right\rangle + \frac{\hat{\Phi}'}{\hat{\Phi}} \left\langle F_s\left(x, K_s\right) \right\rangle \tag{6}$$

where $\left\langle F_p\left(K_p\right) \right\rangle$ and $\left\langle F_s\left(x, K_s\right) \right\rangle$ are the time averaged primary radiation forces of the progressive and the standing wave as of Eq. (1). When the coefficients from Eq. (6) are used in Eq. (1) term III can be calculated as

$$\frac{\left\langle F_p\left(K_p\right) \right\rangle}{\left\langle F_s\left(x, K_s\right) \right\rangle} = (ka)^3 \cdot \frac{K_p(\lambda, \sigma)}{K_s(\lambda, \sigma)} \cdot \frac{\hat{\Phi}_p^2}{2\hat{\Phi}_s^2} \cdot \frac{1}{\sin(2kx)} = \cdots \frac{\hat{\Phi}^2 - \hat{\Phi}'^2}{2\hat{\Phi}\hat{\Phi}'} \cdots \tag{7}$$

This resulting term in Eq. (7) can become rather high for low values of $\hat{\Phi}'$, which means that the influence of the primary radiation force of the progressive wave must not be neglected in such cases.
Furthermore consideration of viscosity might be necessary as Eq. (1) is a low viscosity approximation. Water-rich EtOH mixtures show a substantial increase of viscosity up to 50%($^{v}/_{v}$) EtOH. At 12%($^{v}/_{v}$) EtOH the viscosity of water-EtOH is more than 1.5 times higher than for pure water.
Higher viscosity could lead to an increase of the radiation force, in particular in a progressive wave [13]. At high frequencies (above 1 MHz) and for particles above 1 µm in diameter the direction of the periodic force may change due to viscosity, particles that in the non-viscous case were driven into the pressure nodes are then concentrated in the displacement nodes [1]. The consideration of viscosity might explain as well the re-establishment of the spatial order at high true electrical power input settings as observed due to some unexpected dependence of the (adiabatic) compressibility of the host liquid on the pressure amplitude or acoustic energy density.

Acknowledgements
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