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FOOD PHYSICS AND MICROBIOLOGIC ASPESTS OF ULTRASOND IRRADIATION OF BIOGICAL MATERIALS USED AS FOODSTUFF

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1. INTRODUCTION, BACKGROUND OF THE RESEARCH WORK

Research work aimed at exploring the cell biology and physical interactions of active ultrasound with the material placed in the ultrasound field was started because we wished to interfere in the life activity, e.g., in the survival dynamics of the cell biology systems, and in the physical systems in such a way that no contamination of material type (chemical or biological contamination) can take place. No comprehensive summary of this topic based on system approach was found either in the Hungarian or in the international literature, only research results of specific sections of this area were found.

2. GOALS OF THE RESEARCH WORK

Our main goal was to study the cell biology effects of the mechanic ultrasound waves, the assumed selective cell biology effects of ultrasound, and the most important factors initiating or affecting these effects. Other goals were to achieve the purposeful control of the cell biological effects through the physical parameters, in order to manipulate the life activity of the cells and to study the changes in the parameters of ultrasound that occur in the sound field. The term „selection" means changing the vitality of one or more selected types of cell. During this vitality change the number of the living cells of the selected cell type changes to the required extent as a result of the treatment. Showing the selective effect of ultrasound and exploring the factors affecting this effect can open up new prospects to affect cell numbers in the food processing industry and in the human therapy either as a separate process or combined with other treatments. The possible options for achieving selectivity are separation, deactivation or

stimulation of reproduction. During our work we studied the cell deactivation and reproduction stimulation effects initiated by the ultrasound treatment and besides we investigated the most important physical factors affecting the survival dynamics.

3. MATERIALS AND METHODS

During the experimental work we applied different model materials. The most important ones were a yeast strain, *Saccharomyces cerevisiae,* finely ground dolomite particle size of which was identical with the cell size of the yeast, AND a conditional pathogen bacterium strain, *Pseudomonas aeruginosa* HNCMB170001. The experiments were carried out in a frequency range around 1.1MHz and in the ultrasound output range of 0-12W/cm^2, under identical treatment conditions. Our research covered altogether nine different aspects of the task; several series of experiments were carried out for each area. Three different ultrasound systems were designed and built for the experiments equipped with analogous and digital cell analytic methods and liquid flow systems. For detecting the acoustic phenomena, namely acoustic streaming, standing wave and cavitation, analogous digital and visual systems and methods were developed. Heat effect of ultrasound was studied separately with infra thermometers and thermocouples. Before the experiments we assumed that one of the effects of ultrasound that may initiate biological effects is the generated heat. Different pieces of ultrasound field modeling and simulation software were compared and applied for the purpose of better understanding of the problems mentioned in the goal definition section hereof. Microbiological formulas and equations were applied and reformulated, and physical calculation methods were applied for

evaluating the experimental results and for determining the hardly measurable parameters. We worked out a method for determining the cavitation threshold concentration and the time period needed for the formation of the cavitation for several model materials at different ultrasound output levels. In the experiments carried out at an ultrasound output of 9W/cm^2, we studied the dynamics of the acoustic phenomena formed in the ultrasound field and the effects of these acoustic phenomena on the survival dynamics of the yeast at multiple levels of the cavitation threshold concentrations. Finally, we compared the results obtained under identical conditions for the yeast and the bacterium and draw conclusions for the possible selectivity criteria in relation to the experiments.

4. RESULTS

In the ultrasound systems of flowing liquid type, at ultrasound radiation output levels of 7.5, 9.6, 10.5, and 12W/cm^2 the outputs that were actually entered in the treatment cuvette were 7.37, 9.43, 10.32 and 11.79W/cm^2, respectively. When 50 ml *Saccharomyces cerevisiae* suspension was applied in a concentration of 2-3*10^7/ml at a temperature of 20ºC, the obtained „D" values were 209.36, 108.42, 59.34 and 53.65. The „D" values were reciprocally proportional to the applied output level. In flowing liquid type systems, the output change that was needed to change the „D" decimation interval by one order of magnitude was 11.89 W/cm^2.

When the initial cell concentration was 0,4-2*10^7/ml and the volume of the solution was 50 ml, the ultrasound initiated cell destruction carried out at variable temperature and at ultrasound output levels of 2.07 W/cm^2 and 2.7 W/cm^2 resulted

in „D” values of 100.1, 158.1, 133.7 91 minutes, and 34.9, 30.64, 35.33, 52.2 minutes, respectively.

Due to the self-absorption of the thermocouple, the experiments aimed at studying the effects of ultrasound were carried out by using an infra thermometer. If cells were present in the ultrasound field, the cavitation activity and heat generation decreased but cell destruction was observed at the applied concentration levels.

When studying the acoustic phenomena, we observed a series of interconnected physical phenomena that consisted of the following elements: still liquid, fountain phenomenon, cavitation, acoustic streaming consisting of bubbles and particles, acoustic streaming that sweeps along particles, standing wave, and acoustic streaming consisting of bubbles and particles.

In the applied ultrasound output range of 3-12W/cm^2, the cavitation threshold concentration is increasing consistently. Cavitation threshold concentration ranges for lyophilized yeast, pressed yeast and finely ground dolomite are 2-4.2 g/l, 9.12-12.08 g/l, 0.88-5.12 g/l, respectively. The time periods required for the formation of the cavitation for ground dolomite having an average particle size of 12μm and for lyophilized yeast were 750 seconds and 45 seconds, respectively.

At an ultrasound output of 9W/cm^2, under the conditions of acoustic streaming, standing waves and cavitation, the decimation periods were 160 – 130 seconds, 1500 – 800 seconds and 39 – 150 seconds, respectively. In the ranges of the acoustic streaming and standing waves, the decimation periods were reciprocally proportional to the initial cell concentration of $1.72*10^7$-$5.37*10^7$/ml, while in the cavitation range this period was directly proportional to the initial cell concentration.

„D" values for *Pseudomonas aeruginosa* at ultrasound outputs 9W/cm^2 and 6W/cm^2 were in the ranges of 10056-1205 seconds and 2656-1968 seconds, respectively and the length of this period was reciprocally proportional to the initial cell concentration. In these experiments the applied ultrasound frequency was 1117 kHz and the initial number of germs was in the range of $5.5*10^7$-$1.24*10^7$.

4.2. NEW SCIENTIFIC FINDINGS

4.2.1. I established that in isotherm ultrasound systems of flowing liquid type and in variable temperature ultrasound systems of loading – unloading type the „D" values of *Saccharomyces cerevisiae* are reciprocally proportional to the applied ultrasound output, and in the variable temperature ultrasound systems these "D" values are directly and reciprocally proportional to the initial cell concentrations at ultrasound outputs in the lower and higher output ranges, respectively.

4.2.2. I established that the ultrasound-generated cavitation is responsible for the increased heat generation in water but the biological effects of ultrasound are caused by other factors as well besides heat.

4.2.3. I proved the possibility of the formation of intracellular cavitation by modeling.

4.2.4. I established that the cavitation threshold concentration changes linearly in the output range of 3-12W/cm^2 for each model material and in the knowledge of the threshold concentration and of the time period needed for the formation of the cavitation, the quality of the material could be determined.

4.2.5. I established that the length of the time period needed for the formation of the cavitation changes linearly with the applied particle concentration but it does not change with the output if the cavitation threshold concentration is changed in the same increments.

4.2.6. I established that at an ultrasound output of 9W/cm^2, under the conditions of acoustic streaming and standing waves, the "D" values of *Saccharomyces cerevisiae* were reciprocally proportional to the initial cell concentration, while in the cavitation range they are directly proportional to this cell concentration in the concentration range of $1.72*10^7$-$5.37*10^7$/ml.

4.2.7. I proved that under the same conditions the survival dynamics of the *Saccharomyces cerevisiae* can be monitored more quickly and in a simpler way by the applied analogous cell analytic system than by the manual method.

4.2.8. I established that the „D" values for *Pseudomonas aeruginosa* at ultrasound outputs of 9W/cm^2 and 6W/cm^2 and in an initial cell concentration range of $5.5*10^7$-$1.24*10^7$ were reciprocally proportional to the initial cell concentration.

4.2.9. It can be established that the contrasting survival dynamics showed the bacterium and the yeast under cavitation conditions can prove the opportunity for selective ultrasound treatments that are specific to different species.

4.2.10. New equipment and new tools are the liquid flow type ultrasound system, the variable temperature ultrasound system, the ultrasound generation and detection systems developed for studying the heat effect of ultrasound, the analogous and digital cavitation detection and cell analytic systems.

4.2.11. New scientific methods are the sono-thermograms and the differential sono-thermograms, the basic and auxiliary methods for determining the cavitation threshold concentration, the methods for determining the moment

when the cavitation occurs, the methods used for the simultaneous study of the acoustic phenomena and the biological effects, cell and the analytic methods for the investigation of the survival dynamics of *Saccharomyces cerevisiae* in the ultrasound field.

5. CONCLUSIONS, RECOMMNENDATIONS

5.1. ULTRASOUND TESTS IN LIQUID FLOW AND VARIABLE TEMPERATURE SYSTEMS

In the studied ultrasound systems of flowing liquid type, at ultrasound radiation output levels of 7.5, 9.6, 10.5, and 12W/cm^2 while the outputs that were actually entered in the treatment flow-through cuvette were 7.37, 9.43, 10.32 and 11.79W/cm^2, respectively. This decrease in the ultrasound intensity was caused by reflection. We suggest that during any type of ultrasound treatment, the main physical parameters are worth stabilizing at steady levels or, if this is not possible, the ultrasound field modifying effects of the most important physical parameters shall be known and shall be taken into consideration when interpreting the results of the experiments.

Both in isotherm ultrasound systems of flowing liquid type and in variable temperature ultrasound systems of loading – unloading type, the „D" values of *Saccharomyces cerevisiae* are reciprocally proportional to the applied ultrasound output, which is caused by the higher biophysical fracturing effect of the higher intensity ultrasound.

5.2. HEAT EFFECT OF ULTRASOUND

Compared to the clear suspending media, the intensity of both the acoustic cavitation and the temperatures were lower. It can be seen from the results that the

ultrasound-generated cavitation is responsible for the heat generation in liquids. Temperature that seems to be independent of the changes of the acoustic phenomena can be observed only in the final phases of the experiments; however, in this zone there was no measurable difference between temperature difference values in the suspension samples of different concentrations and the clear suspending agent either. We suggest that the dynamics of the acoustic phenomena shall be studied for every acoustic system to be treated that is for every material to be examined, because this is essential for all work that is related to active and passive ultrasound.

5.3. MODELING THE ULTRASOUND FIELD

Results of the model experiment show that above a certain acoustic pressure amplitude (accurate level of this acoustic pressure amplitude can be different in each acoustic system due to the wave modifying effects prevailing in the specific system) intracellular cavitation may occur, as the ultrasound beam is concentrated by the concave surfaces that have acoustic hardness different from the acoustic hardness of the other zones. We suggest that the modeling the ultrasound field and the experimental work shall supplement each other for the purpose of the preparation of the experiments and of interpreting the results.

5.4. INVESTIGATION OF THE ACOUSTIC PHENOMENA

At the applied ultrasound output range of 3-12 W/cm^2 the cavitation threshold concentrations for the lyophilized yeast and for pressed yeast were 2-4.2g/l, and 9.12-12.08g/l, respectively. When recalculating the results obtained for

the two different forms of yeast to the dry matter content on a wet material basis, very similar cavitation threshold concentrations are obtained for each output levels. From this fact we draw the conclusion that the cavitation threshold concentrations is basically depends on the dry matter content of the individual materials.

The moments when cavitation is formed in the cases of ground dolomite and lyophilized yeast are about 750 seconds and 45 seconds, respectively. The difference may be caused by the different inertia of the particles having different density and movement. As a consequence, it can be stated that the materials can be characterized qualitatively and quantitatively in a repeatable manner with their respective cavitation threshold concentrations and with the exact points in time when the cavitation occurs. We suggest that the conditions and physical criteria of the presence of the different acoustic phenomenon in the ultrasonic field shall be taken into consideration and we also suggest using the above methods as particle analytic methods or quick methods for determining the dry matter content of the different materials.

5.5. EVALUATION OF THE SURVIVAL DYNAMICS OF THE YEAST *SACCHAROMYCES CEREVISIAE* BY CONSIDERING THE ACOUSTIC PHENOMENA

In case of suspensions with higher initial concentrations of *Saccharomyces cerevisiae,* formation of the standing wave after the acoustic streaming and the cavitation after the standing wave occurs later as a result of the lower acoustic pressure due to the higher absorption and reflection of ultrasound by the particles. In the ranges of acoustic streaming and standing wave, and in the cavitation range, the decimation periods are reciprocally proportional and directly proportional to

the initial cell concentration, respectively. There is an interaction between the suspension concentration in the ultrasound field and the formation of the acoustic phenomena and consequently, between the survival dynamics of the cells in the suspension and the suspension concentration. We suggest that the acoustic phenomena that determine the survival dynamics shall be influenced through the physical parameters of the ultrasound field. By this way the acoustic phenomena determining the survival dynamics can be influenced through the physical parameters of the ultrasound field. In this way the survival dynamics can be controlled by the cell concentration itself through a feedback loop.

5.6. APPLICATION OF THE CELL ANALYTICS METHODS

By using the applied analogous cell analytic method, the survival dynamics of the *Saccharomyces cerevisiae* could be monitored more quickly and in a simpler way than by applying the manual vital dyeing method. We recommend the use of the applied analogous cell analytic method in determining the ultrasound resistance of the cells. By using this method indirect information can be obtained on the distribution of a cell population, on the species composition of a system and, in the area of environmental analytics, on the toxicity or mutagen effects of a given system.

5.7. ULTRASOUND TREATMENT OF *PSEUDOMONAS AERUGINOSA* BACTERIUM

„D" values of the *Pseudomonas aeruginosa* bacterium were reciprocally proportional to the initial cell concentrations at both 6 and 9W/cm^2 ultrasound output. Consequently, in the applied concentration range the system could not

achieve its peak capacity that is the cavitation threshold concentration, as at higher concentrations the collapse of a cavitation bubble may destroy more than one cells in the vicinity of the bubble. Lower intensity ultrasound, if applied for a short period of time, stimulated the reproduction of the bacteria. We recommend operating the system near the cavitation threshold concentration, but below this concentration level if the goal is to destroy the examined bacteria or all the microorganisms. In this case the operation is done below the safe upper limiting value of an acoustic phenomenon at the stable peak capacity. The cavitation threshold concentration can be determined by an experiment. For stimulating the reproduction, low intensity ultrasound shall be applied for short time intervals.

5.8. CRITERIA FOR THE SELECTIVE ULTRASOUND EFFECT

In case of the *Saccharomyces cerevisiae* in the cavitation range the decimation interval is directly proportional to the initial suspension concentration, while in case of the *Pseudomonas aeruginosa* bacterium these two parameters reciprocally proportional to each other. If the initial number of germs is $9.22*10^7$/ml, for both of the microorganisms, then theoretically the decimation interval for each of them is the same, 737 seconds, and when subjected to ultrasound treatment, they are destroyed at the same rate. If the initial cell concentration is lower than the aforementioned concentration, the yeast can be eliminated from the suspension containing both of them. In the reverse case the bacteria can be exterminated while the yeast remains. Selectivity of the output is practically unidirectional as the „D" value of the yeast is approximately one tenth of the „D" value of the bacterium. This means that the reverse case can only exist if the concentration of the yeast is higher by at least ten orders of magnitude that

the concentration of the other organism. If the subject bacteria shall be exterminated while the yeast shall be retained in a case where its initial number of germs , or its „D" value is lower, the reproduction stimulation effect at an ultrasound output of 6W/cm^2 shall be applied (if needed, in more than one phases) for multiplication of the bacteria so that its „D" value shall be lower than the yeast and in such a way it can be eliminated from the yeast containing solution. Consequently, ultrasound is suitable for the selective control of the number of cells, so we recommend its use for selective cell biology treatments even in case of other species.

6. PUBLICATIONS

6.1. LECTURE ARTICLES

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2. Lőrincz, A. – Neményi, M. (2002): Akusztikai kavitáció kialakulásának koncentrációfüggése szuszpenziókban. Élelmiszerfizikai közlemények. (accepted)

3. Lőrincz, A. – Neményi, M. (2002): Examination of the concentration dependence of acoustical phenomenon in water based suspensions. Acta Agronomica Ovariensis. (accepted)

4. Lakatos, E. – Lőrincz, A. – Neményi, M. (2002): Az ultrahangos sejtroncsolás fizikai kritériumainak meghatározás a folyékony élelmiszerek csíraszám csökkentésével kapcsolatban. Élelmezési Ipar. LVI. Évfolyam 2002. 7. Sz. pp. 203-206.

5. Lőrincz, A. – Neményi, M. (2002): Appreciation of an complex ultrasound system according to survival cell count. Hungarian Agricultural Engineering. (accepted)

6. Neményi, M. – Lőrincz, A. (2002): Analysis of the concentration dependence of ultrasonic cavitation in suspensions by considering particle analysis and particle manipulation factors. Ultrasonics (under the process)

7. Lőrincz, A. (2003): Effectiveness of ultrasonic cell disruption as a function of the suspension concentration. Acta Alimentaria (accepted)

8. Lőrincz, A. (2003): Analysis of the concentration dependence of ultrasonic cavitation in food industrial water based suspensions and its connection with the ultrasonic cell disruption. Biosystems Engineering (under the process)

6.2. PROPAGATE ARTICLES

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10. Lőrincz, A. – Neményi, M. (2002): Az in vitro sejtfeltárás hatékonyságát befolyásoló fizikai tényezők (2. rész). Laboratóriumi Információs Magazin XI. évfolyam, 2002/3. szám, Biofizika rovat. Pp. 33-35.

6.3. PRESENTATIONS

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12. Lőrincz, A. - Neményi, M. (2001): Cell decrease by ultrasonic effect on yeast (*Saccharomyces cerevisiae*) suspension and the limit concentration of cavitation. 08. 28-30. Phisical Methods In Agriculture, Prága (oral presentation +poster)

13. Neményi, M. – Lőrincz, A. (2001): Cell concentration decreasing with ultrasonic effect of yeast (Saccharomyces cerevisiae) suspension. Műszaki Kémiai Napok, Veszprém. (poster)

14. Neményi, M. – Lőrincz, A. (2001): Cell (*Saccharomyces cerevisiae*) disruption with ultrasound treatment. In: Institute of agricultural, food and environmental engineering. Conference für Leben und Überleben, Internationaler Kongress, Wien, Universitat für Bodenkultur, November 18-21. p. 192. (poster)

15. Neményi, M. – Lőrincz, A. (2002): Komplex ultrahangrendszer értékelése a besugárzás miatt kialakult mikroorganizmus-csíraszám csökkentő hatás alapján. MTA-AMB Kutatási-Fejlesztési Tanácskozás, Gödöllő, Nr. 26, p. 38. (poster)

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17. Neményi, M. – Lőrincz, A. (2002): Különböző típusú szuszpendált szemcsék tulajdonságainak hatása az ultrahangos kavitációra. Műszaki Kémiai Napok, Veszprém, 2002. április 16-18. pp. 260-261. (oral presentation +poster)

18. Neményi, M. – Lőrincz, A. (2002): Az ultrahang sejtbiológiai hatásinak elemzése a hangtér fizikai paramétereinek függvényében. XXXII. Membrán-Transzport Konferencia. A Romhányi György Alapítvány, A Magyar Élettani Társaság Membránbiológiai Szakosztály és a Magyar Biofizikai Társaság közös rendezvénye. Sümeg, 2002. május 21-24. (oral presentation +poster)

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22. Neményi, M. – Lőrincz, A. – Lakatos, E. (2003): Az ultrahangsugár fizikai paramétereinek változása a besugárzott anyagban. MTA-AMB Kutatási - Fejlesztési Tanácskozás, Gödöllő, 01.21-22, Nr. 27, p. 55. (poster)

23. Lőrincz, A. – Neményi, M. – Lakatos, E. (2003): A magas intenzitású ultrahang sejtroncsoló hatásának alakulása a besugárzott anygtól függő akusztikai jelenségek mellett. MTA-AMB Kutatási-Fejlesztési Tanácskozás, Gödöllő, 01.2-22, Nr. 27, p. 79. (poster)

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