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Biotechnology

ULTRASONIC STIMULATION OF *CHLORELLA* POPULATION GROWTH

ULTRADŹWIĘKOWA STYMULACJA WZROSTU POPULACJI *CHLORELLA*

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Ultrasonic (high frequency/low intensity) stimulation of *Chlorella vulgaris* is shown to intensity population growth. The effect is caused by a change in the cellular membrane potential as a result of increased permeability of the membrane; metabolic rate is thus determined by the cell's energy demand.

It is suggested that sonication can be applied in biotechnological processes involving *Chlorella*.

INTRODUCTION

The concept of virtually waste-free production as well as certain technologies in fish processing industry make it possible to utilise raw materials almost completely, to process the by-products and wastes, and to reduce pollution of the environment.

Improved equipement and technologies allow to extend the scope of waste utilisation and are conducive to proposing novel applications.

Biotechnological processes, effected by both living cells and certain biologically active substances extracted from them (e.g., enzymes contained in the cells) provide an excellent tool with which to process industrial wastes.

The greatest potential is offered by taking advantage of processes that normally operate within living cells of microorganisms. However, a large-scale application of those processes is problematic due to their relatively low rates. To increase the rate of a biotechnological process calls for intervention into metabolic regulation. This can be effected in different ways. However, there has been so far no simple and versatile industrial-scale method whereby microorganisms could be stimulated to increase the rate of processing wastes and sewage as their substrates and to perform supersynthesis.

Increased permeability of the cytoplasmic cellular membrane is the basis of physiological metabolic regulation, whereby supersynthesis is achieved (Vorobieva 1989); as a rule, this is the process that determines the rate and extent of release of substances.

Ultrasonic treatment (sonication) is one of the ways in which cytoplasmic membrane permeability can be increased, thus intensifying development of a microorganism. Ultrasonic stimulation of exchange processes in microbial cells has already been used for medical, veterinary, and pharmaceutical purposes. Sonication applied to microbial population has a great potential for intensifying biotechnological processes. Results of studies can be viewed from a systemic level, whereby a system is regarded as an active transformer of matter and energy; there is no need to revert to interaction between individual components of the system.

From a practical point of view, ultrasonic stimulation of the microalga *Chlorella vulgaris* populations in order to intensify their rate of sewage treatment in a fish processing plant is of a great importance.

MATERIAL AND METHODS

Chlorella vulgaris is present under natural condition in the Tamja. In the experiment, population densities of 14×10^6 cells ml^{-1} similar to the natural ones, were used.

The algae were cultured in connection with developing a complex sewage technology to be used in a fish processing plant. An ultrasonic generator (880 kHz, intensity range of 0.1×10^4 to 1.0×10^4 Wt m^{-2}, sonication time of 1 to 10 min) was used in the experiment.

Algal cultures were kept in 500 cm^3 flasks; no mixing was apllied to those microcosm which were allowed a free gas exchange with the atmosphere. The temperature was kept at a stable level of 26±1°C with an ultrathermostat. Intensification of biological processes in the population was assessed from changes in population density, respiration, and rate of photosynthesis. Population density was estimated from counts made in the Bürker chamber. Rates of respiration and photosynthesis were determined concurrently, in the light and in darkness, using an oxymeter.

Inorganic phosphorus contents were determined colorimetrically (Privezencev 1973) after protein precipitation with chloroacetic acid. Changes in the *Ch. vulgaris* population were determined using infrared spectroscopy (Lukanev et al. 1980).

RESULTS AND DISCUSSION

Analysis of the data pertaining to the population growth dynamics of *Ch. vulgaris* shows changes in the course of S-shaped curves describing the population growth following sonication. The changes may be divided into 5 stages (Fig. 1). Increase in the sonication intensity up to 0.6×10^4 Wt m^{-2} did not affect the shape of the curve (stage 1). A subsequent increase up to 0.8×10^4 Wt m^{-2} brought about a shortened lag phase, but the population

density remained virtually unchanged (stage 2). At the sonication intensity of 1.0×10^4 Wt m^{-2}, the lag phase was no longer shortened, the population density increased by the factor of 1.5 and the expotential phase was elongated by a factor of 1.3 (stage 3). Following the sonication intensity increase to 1.2×10^4 Wt m^{-2} the lag phase duration increased 1.8 times relative to the control, the population density increasing 1.2 times. It should be mentioned that under such experimental conditions the system is subject to an „acoustic shock" which intensifies biomass accumulation (stage 4). At the sonication intensity exceeding 1.2×10^4 Wt m^{-2}, the culture was showing symptoms of destruction (stage 5).

The inorganic phosphorus uptake increased during the lag phase only at stage 2. The increase continued until the expotential phase at stage 3, while a decrease during the lag phase and an increase during the exponential phase were observed at stage 4. The uptake decreased at stage 5.

Analysis of the oxygen release dynamics, regarded as a measure of gross photosynthesis rate, showed that the rate practically did not differ during stages 1 to 4 from the control rate. At stage 5, the gross photosynthesis rate plummeted throughout the entire duration.

Chemical composition of the sonicated and non-sonicated populations showed the protein content in the first, while subject to the non-destructive treatment, to increase by the factor of 3 at the intensification stage and to double at the „acoustic shock" stage, compared to the control.

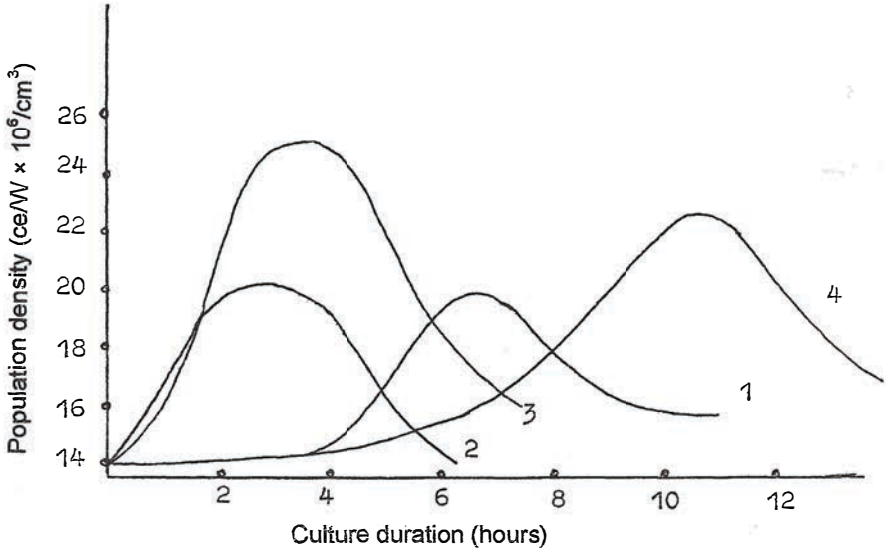

Fig. 1. S-shaped curves showing changes in *Chlorella vulgaris* population density under sonication of different intensity
1 - Control - 0.6 10^4 Wt m^{-2}; 2 - 0.8 10^4 Wt m^{-2}; 3 - 1.0 10^4 Wt m^{-2};
4 - 1.2 10^4 Wt m^{-2}.

Infrared spectra of the sonicated and non-sonicated *Ch. vulgaris* populations show absorption curves to be situated at 1500 - 1800 cm^{-1}, which is characteristic of peptides, and at 1600 cm^{-1}, typical of amide I (valent vibrations CO). As reported by Lukanev et al. (1980), nucleic acid absorption in the amide I curve is very low. Increase in the realtive intensity of the long-wave component (amide I curve) at about 1630 cm^{-1} indicates protein transformation into B form (Čirgadze 1965; Palm 1969).

In those parts of the spectrum there is no increase in the relative intensity of partial absorption in the sonicated cells. On the other hand, sonication intensities leading to the „acoustic shock" and cell destruction produced marked differences in the spectra, more pronounced in the parts associated with the sonication intensity that causes cell destruction that in those related to the „acoustic shock" - producing intensities.

Analysis of different spectra showed also a decrease and an increase in absorption, observed at 1550 - 1660 and at 1630 cm^{-1}, respectively. This is evidence of changes occurring in various forms of polypeptid chain configuration and destruction of the protein spatial structure.

Analysis of lipid infrared spectra produced by the samples failed to detect any marked differences between various sonication intensities. Absorption was observed to increase at 1065 cm^{-1} (monoglycerides) and at 1710 cm^{-1} (carbonyl compounds) under the „acoustic shock" treatments: absorption increased at 3560 cm^{-1} and decreased at 3012 - 3020 cm^{-1} (unsaturated fatty acids) (Fig. 2).

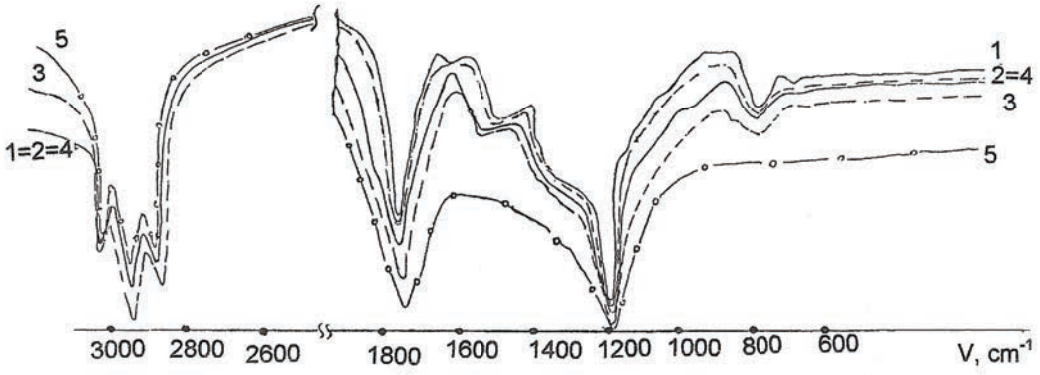

Fig. 2. Lipid infrared spectrum of *Chlorella vulgaris* subject to sonication of different intensity
1. - to 1.0×10^4 Wt m^{-2}; 2. - 1.2×10^4 Wt m^{-2}; 3. - 1.4×10^4 Wt m^{-2};
4. - 1.2×10^4 Wt m^{-2} (a day after treatment); 5. - 1.4×10^4 Wt m^{-2} (a day after treatment).

Similar assays ran during the daytime showed the changes in those cells subject to the „acoustic shock" to be almost completely absent. The cellular protein affected by the ultrasonic treatment of intensity in excess of 3000 kJ m^{-2} during the daytime underwent further

destruction; lipids were brought to further oxidation and hydrolysis (increase in the absorption intensity curve at 3560 cm^{-1}).

Ultrasonic spectroscopy analyses showed that those treatments intensifying growth of the microalga produced no change in proteins and lipids of the sonicated populations of *Ch. vulgaris*. Changes brought about by the treatments leading to the „acoustic shock" are small, too, and involve partial protein denaturation and increase in the lipid peroxide group. In those treatments leading to destruction cells, irreversible denaturation of protein occurred, followed by protein hydrolysis and oxidation and hydrolysis of lipids.

The present study shows that the *Ch. vulgaris* population sonicated with intensities of 0.8×10^4 to 1.0×10^4 Wt m^{-2} increased its inorganic phosphorus uptake, the increase being typical of higher ATP activity.

The sonication intensity of 1.2×10^4 Wt m^{-2} produces some destruction in the natural structure of protein. This is turn intensifies protein synthesis as the cell is stimulated to compensate for the loss. Sonication seems to enhance biological sewage treatment with the use of *Ch. vulgaris* in fish processing plants. Sonicated and non-sonicated populations used in sewage treatment technologies showed marked differences in their nitrogen reduction capacities(0.5 and 2.0 mg/dcm^3,respectively); amino acid content decreased by a factor of 1.5 (0.06 and 0.09 mg/dcm^3, respectively) while a 2.5-fold reduction in nitrate nitrogen (0.01 and 0.1 mg/dcm^3) and a 3.5-fold one in nitrite nitrogen (0.08 and 0.28 mg/dcm^3, respectively) were recorded as well.

The positive effect of low intensity sonication is due to the fact that a cell affected is mobilised to use its reserves for growth and development. The effect is sufficient to affect biological processes of regulation and compensation, without demaging reversible reactions (Akopjan et al. 1988).

The effect is caused by microflows in the cell and around it and an increase in the cellular membrane permeability, which changes the membrane potential.

The change in membrane potential in turn accelerates ATP synthesis and intensifies biological processes. Destruction and reduction in mitochondrial membrane affects the membrane's non-specific conductivity for H^+ or produces a complete disappearance of the membrane, in which case the ATP activity has to increase. It can be concluded that a rapid and reversible ATP synthesis can take place in an active centre without energy expenditure on the part of the hydrogen ion electrochemical potential. This is the process the rate of which increases 1000-fold once the membrane energised (Anisimov et al. 1986), i.e., when its potential increases, which takes place following increase in cellular membrane permeability due to sonication. Metabolic rate is controlled by the cell's energy demand rather than by contents of various substances in the medium.

This study demonstrates a possibility to use low intensity sonication to intensify physiological processes and population growth of *Ch. vulgaris*, which can be applied in biological sewage treatment in fish processing plants.

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ULTRADŹWIĘKOWA STYMULACJA WZROSTU POPULACJI *CHLORELLA*

STRESZCZENIE

Na przykładzie *Chlorella vulgaris* wykazano, że poddanie jej działaniu ultradźwieków o wysokiej częstotliwości intensyfikuje wzrost ilości komórek w populacji.

Efekt intensyfikacji związany jest przede wszystkim ze zmianą potencjału membrany komórki w wyniku zwiększenia jej przepuszczalności, tzn., że szybkość procesów metabolicznych określana jest przez zapotrzebowanie energetyczne komórki.

Wyrażone jest przypuszczenie, że ultradźwięki o powyższej charakterystyce mogą znaleźć zastosowanie w procesach biotechnologicznych z udziałem *Chlorelli*.

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