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Release of Polysaccharide by Sonication of Cells (*Porphyridium sp.*)[1]

V. Faerman, I. Mukmenev, and I. Shreiber

Department of Chemical Engineering, Ben-Gurion University of the Negev, P.O.B. 653, Beer-Sheva, Israel

e-mail: faerman@bgu.ac.il

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The paper deals with experimental data concerning the interaction of acoustical waves with microbial cell *(Porphyridium sp.)*. The aim of the present paper was to increase the amount of biopolymer released from the microorganisms biomass with the aid ultrasound irradiation without scission or a decrease in the molecular weight. The results indicated that the amount of polysaccharide (for example) released from the cell pellet could be enhanced by ultrasound, depending on the frequency and energy of the ultrasound. The sugar composition remain the same, but the apparent viscosity of polysaccharide aqueous solutions decreased, indicated that some changes in the molecular shape and size occurred. When ultrasound irradiation was applied in the presence of either CO_2 or $CO_2 + H_2$, the apparent viscosity of polysaccharide aqueous solutions increased (versus usual ultrasound treatment).

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BRIEF BACKGROUND

It is known that nature of a gas saturating a liquid medium will influence an ultrasonic chemical reaction [1]. Several investigation have shown that the action of ultrasonic waves on macromolecules leads either to depolymerization or polymerization processes [2–4].

Depolymerization of macromolecules is due to cavitation effects, e.g., in polystyrene in toluene as well as in hydroxyethyl cellulose in water at a frequency 175 kHz [1], the molecular weight of the irradiated polymer was reduced to 0.1 of its initial value. There was no reduction in molecular weight in polymer irradiated in a degassed liquid, in which cavitation is greatly inhibited. An investigation of the effect of ultrasound on polystyrene in toluene and carboxymethyl cellulose in water at a frequency of 960 kHz and intensity of 6.8 W/cm^2 gave similar results [1]. In the latter study, depolymerization of the long-chain molecules took place when the irradiated liquid was saturated with gases promoting cavitation (for example O_2). In a degases liquid or in a liquid saturated with carbon dioxide, no depolymerization occur.

MICROORGANISMS AND GROWTH CONDITIONS

Microalgae [*Porphyridium sp.*] were grown [5] at 25 ± 0.5°C in glass columns filled with artificial seawater. Illumination was supplied by a bank of fluorescent lights at an intensity of 150 microeinstains/m^2/sec.at the surface of column.

The cultures were aerated simultaneously with air at a rate of 0.15 l/min and CO_2 at a rate of 0.01 l/min.

ULTRASOUND EQUIPMENT

Ultrasound energy was provided at two different frequencies by using two kinds of ultrasonic devices:

1. At 20kHz (consumable power 450 W, continuous mode) by a Vibra Cell 450 (Sonic and Materials, Danbury, CT).

2. At 40 kHz (consumable power 50 W, continuous mode) by an ultrasonic device (Fig. 1), which elaborated based on magnetostrictive ferrite radiator of ultrasound [6–8] of Acoustics Institute [9, 10].

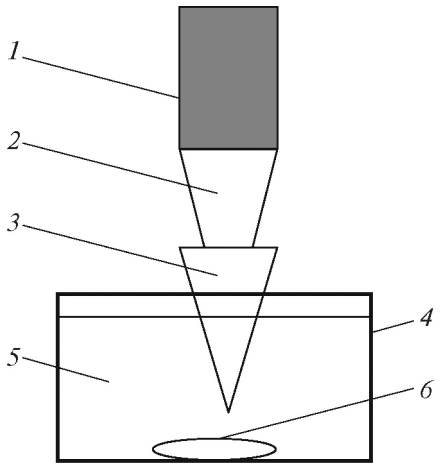

Fig. 1.

[1] The text was submitted by the authors in English.

EXPERIMENTAL DEVICE

Ultrasound treatments were performed in a specially designed (Fig. 1) stirred chamber (*4*) including transducer, containing ferrite radiator (*1*), ultrasound concentrator-first step (*2*), second step (*3*), tube CO_2—not shown, tube H_2—not shown, water jacket—not shown, stirrer (*6*) and microorganisms suspension (*5*).

Polysaccharide was measured as total released carbohydrate (Phenol Acid Method) [11].

RESULTS

The results indicate clearly that the amount of polysaccharide released from the cell pellet could be enhanced by ultrasound depending on the frequency. Quality of the solved polysaccharide was 2.25 mG/ml at the frequency of 20 kHz and 1.75 mG/ml at the frequency of 40 kHz. At the same time the chemical composition of the suger was the same (see Table 1).

However, the apparent viscosity of polysaccharide aqueous solutions decreased about 37%, indicating that some changes in the molecular shape and size occurred. When ultrasound was applied in the presence of either CO_2 or $CO_2 + H_2$, the apparent viscosity of polysaccharide aqueous solutions decreased only by 10%. Clearly, the incorporation of the gas during ultrasound irradiation prevented significant changes in the physical properties of polysaccharide and indicate to enhance of polysaccharide released from pellet by ultrasound in the presence of CO_2 and increase viscosity simultaneously (versus conventional ultrasound treatment) (Tables 2 and 3).

The viscosity of an aqueous polysaccharide solutions was measurement with an Ostwald viscometer.

The relative viscosity was thus:

$$\eta_{us} = 1.370 \pm 0.001; \eta_{us+CO_2} = 1.473 \pm 0.004.$$

DISCUSSION

The cells of the red microalgae *Porphiridium sp.* are encapsulate within a sulfated polysaccharide. During growth, the viscosity of the medium increase due to the release of extracellular polysaccharide from the cell-wall surface. To enhance the release of polysaccharide from the cell wall without damage to size and properties, we have used ultrasound irradiation, which is known to change the permeability of cell wall probably by removal of extracellular biopolymers selectively [12].

The amount of polysaccharide released from the cell pellet could be enhanced by ultrasound depending on the frequency due to the cavitation. Bubble cavitation shear off the extracallular polysaccharide layer and improves nutrient and oxygen transfer into the polysaccharide elaborating cells. The bubbles obtained at 20 kHz are the most effective since they correspond in their size to that of cells surrounded by polysaccharide layer. Proposed mechanism of phenomenon may be explained by

Table 1

Sugar	Non-treated (%)	Ultrasound treated
Xylose	47	45
Galactose	27.9	30
Glucose	24.8	23

Table 2. Analyses of the polysaccharide after treatment with ultrasound and ultrasound + CO_2

Samples	Polysaccharide (mg/ml)
Control	2.00 ± 0.1
Ultrasound	6.72 ± 0.4
Ultrasound_CO_2	8.75 ± 0.6

Table 3. Results of viscosity measurements with an Ostwald viscometer

Outflow time for doubly distilled water (sec)	Outflow time for the polysaccharide solution subjected to ultrasound (sec)	Outflow time for the polysaccharide solution subjected to ultrasound and CO_2 (sec)
66.76 ± 0.06	91.47 ± 0.06	98.36 ± 0.3

Minnaert's formula for resonance frequency bubble cavitation [13]:

$$f_{res} = (1/\pi d)\,(3\zeta P_0/\rho)^{1/2}, \quad (3)$$

where d—diameter of bubble, ζ—ratio heat capacity of gas in a bubble, P_0—hydrostatic pressure in liquid.

For air bubbles in water at $P_0 = 1$ atm, $\zeta = 1.14$ and $\rho = 1$ – obtained:

$$f_{res} = (0.657/d)\ \text{kHz}, \quad (4)$$

Frequency	Bubble radius	Effective size of cells with polysaccharide layer
20 kHz	about $R = 160$ micron	about 150 micron
40 kHz	about $R = 80$ micron	about 150micron.

The increase of polysaccharide yield was found to correlate the size of cavitation bubbles.

The frequency dependence of polysaccharide production may be explained and be confirmed too by the phenomenon of cavitational erosion [14], which described:

$$K = R_{max}^3 / (R_{min}^3 \Delta t f), \quad (5)$$

where K—erosion activity, R_{max} and R_{min}—maximal and minimal radiuses of bubble, Δt—time of collapse, f—frequency.

In addition to the aforementioned mechanism of cell decay, other cell decay mechanisms are possible, which may be related to pressure gradients, local heating to high temperatures, giant accelerations of ultrasound propagation under cavitation, and chemical effects of ultrasound [15, 16].

CONCLUSION

—Polysaccharide production can be enhanced by periodical ultrasound treatment depending on the frequency, intensity and duration.

—When ultrasound irradiation was applied in the presence of either CO_2 or $CO_2 + H_2$, the apparent viscosity of polysaccharide aqueous solutions increased (versus usual ultrasound treatment) and, hence, prevention drastic decrease of molecular weight of polysaccharide due destruction.

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