

Preparation and characterization of composite hydrocolloid films from sodium alginate and high methoxyl pectin

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A technology for obtaining of a composite biodegradable film on the basis of sodium alginate and apple high methoxyl (HM) pectin has been developed. Good mechanical characteristics have been established when using the two polysaccharides in proportion 1:1. For them the ultimate tensile strength (TS) was 53,53 MPa, the elongation at ultimate strength (E) was 34,48%, Young's modulus (YM)- 202,84 MPa. The composite film showed very poor solubility at low pH, including in a simulated gastric juice. At pH values above 4,0 the alginate films showed slower and the composite films faster and complete disintegration in the medium. Due to the good mechanical characteristics and selective solubility depending on pH, the composite sodium alginate-HM pectin films are appropriate as material for encapsulating of biologically active substances or microorganisms with low activity or survival, under conditions of low pH.

Key words: composite films, alginate, high methoxyl pectin, mechanical properties, dissolution

INTRODUCTION

A wide range of biopolymers are used as a base for films, coatings and therapeutical systems with application in medicine, pharmacy and food industry. Very often the obtained biopolymer systems represent a monohydrocolloid layer of proteins or polysaccharides. During the last several years, however, the technological investigations have been directed towards the development of composite films, which are obtained by combining of proteins, polysaccharides and/or lipids. They are designed with the aim of using the synergetic effect of the combined properties of the separate components [1].

The salts of alginic acid and the pectins are an example of polysaccharides with good film forming properties. The alginates are hydrophilic polysaccharides, extracted from various species brown seaweed (*Phaeophyceae*). They are of interest because of their unique colloid properties and their capacity to form tridimensional networks in the presence of polyvalent cations (for example Ca^{2+}) in water medium as a result of which a gel or an insoluble polymer are obtained [2,3]. By chemical structure the alginates are unbranched binary copolymers, built mainly of (1-4)-linked β -D-mannuronic acid and α -L-guluronic acid residues. There are three types structural elements in the alginates: β (1-4)-D-mannuronate (M-block), α -(1-

4)-L-guluronate (G-block), and the third structure contains both monomers in almost equal proportions (MG-block)[2,3,4]. From the alginic acid salts the sodium alginate is most frequently applied in practice.

The pectins are plant polysaccharides. Their primary structure is a branched chain, in which the main chain consists of acid derivatives of the hexoses (D-galacturonic acid) and the side chains are built of pentoses(D-xylose and L - arabinose) and hexoses (D – glucose and D - galactose). In the main chain the units D-galacturonic acid are linked by α -(1-4) glycoside link, i.e. it is a polygalacturonic acid to which side chains are attached by β -(1-6) glycoside links. The carboxyl groups of the galacturonic acid are partially esterified with methanol and neutralized with metal and ammonia ions obtaining different pectin substances – pectins, pectates and pectic acids [5,6].

Between the full methylation and the complete demethylation of the polygalacturonic acid there are all intermediate stages of partial esterification which results in a great variety in pectins from different raw materials. It is known that the basic index, exerting the greatest effect on the rheological and physicochemical properties of pectin is the degree of esterification (DE) by which is expressed the percentage of the esterified $-\text{COOH}$ groups from the total number. Depending on the degree of esterification pectins are divided into 2 main categories – high methoxyl (HM) and low methoxyl (LM) pectin, and the low methoxyl

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pectin is subdivided into 2 groups – conventional and amidated. The degree of esterification of the HM pectins varies from 50 to 90%. The LM pectins (DE under 50%) are usually obtained by demethylation of the extracted pectins through enzyme processes or by treating with acids or ammonia in alcohol medium. HM and LM pectins have a different mechanism of gel forming when dissolved in water [7,8].

The wide use of alginates and pectins in the food industry and medicine is dictated by the lack of toxicity and allergenicity, and the unique colloid properties. Both biopolymers are capable to form gels in the presence of divalent cations [4,9]. The alginate and the pectin form synergic mixed gels which lead to structures completely different from those of the pure biopolymers. They are of particular interest when creating films with new improved characteristics.

The nature of the synergy interaction between pectin and alginate has not been fully clarified. The rheological behavior of the mixed solutions and hydrogels has been studied to a great extent [4, 10, 11], while the investigations in the field of the physicochemical properties of the resulting composite films are less developed.

The objective of the present investigation is the preparation of a composite biodegradable film on the base of sodium alginate and apple high methoxyl pectin, and assessment of the physicochemical and mechanical characteristics of the obtained materials.

EXPERIMENTAL

Materials: Apple high methoxyl pectin (CpCelso), sodium alginate (Sigma Aldrich), glycerol p.a. (Merck), calcium chloride p.a. (Merck).

Biopolymer films preparation: Sodium alginate (2,5%) and high methoxyl pectin (2,5%) were dissolved in distilled water. Film forming compositions were prepared by casting sodium alginate and high methoxyl pectin aqueous solutions at the following proportions: 100–0% (AG), 75–25% (C₁AG-P), 50–50% (C₂AG-P) and 25–75% (C₃AG-P). Glycerol was used as a plasticizer (0.6g/g polymers). Four formulations were mixed under continuous stirring (300rpm) until a homogeneous solution was obtained, then the stirring rate was reduced to 100 rpm while 0.1 M CaCl₂ (1ml/100ml) was added. The prepared film forming solutions (FS) were poured onto Petri dishes (0,325 g FS/cm²) and were dried under vacuum (20 kPa, SPT-200 Vacuum Drier) at 35°C.

Dried samples were immersed for 30 minutes in 10 ml of 0.3 M CaCl₂ solution to allow cross-linking, and were washed with distilled water to remove excess Ca²⁺. Films were dried at 25°C and conditioned at 50 ± 1% RH before testing.

Analysis of the source pectin: The content of methoxy-groups, galacturonic acid and the DE was determined by the method described in the monograph for pectin in USP 23 [12].

Moisture content - by express weight method with infrared dryer (Sartorius Thermo Control YTC 01 L).

Determination of pH - pH-meter 3310 (Jenway).

Rheological tests - rotational viscometer (Reomat 108), at temperature of the investigated mixtures 20 ± 1°C.

Film thickness: The thickness of the films was measured with a digital micrometer to the nearest 0.01±5% at five random locations on the film. Mean thickness values for each sample were calculated and used in mechanical properties determination.

Mechanical properties: Mechanical properties were determined according standard BDS EN ISO 527-2:2002 [13] with apparatus UMT: Macro mechanical and tribology tester (CETR-USA). Investigations were made for: strength at the moment of destruction (*Ultimate Strength, TS*); modulus of the elastic deformations (*Young's modulus, YM*); strength at plastic deformation (*Yield strength*) and elongation at destruction (*Elongation at Ultimate strength, E*). The results are presented as average values of 6 measurements for each sample.

In-vitro dissolution tests: Model systems, simulating the conditions in the digestive tract were used. Discs from the trial series (with area 1,77 cm²) were put in simulated gastric juice (pH 2,0; pepsin) and simulated intestinal juice (phosphate buffer pH 8,0; enzyme complex - pancreatin), prepared according to Charteris et al.[14].

Additionally a test was carried out with simulated intestinal juice in Tris-buffer medium with pH 8,0. The changes in the samples were recorded at every hour as a percentage alteration of the area. The results are presented as the mean of three replicates.

Test for dissolution depending on pH. The effect of pH on the disintegration of the different film variants (alginate and alginate-pectin) was followed up. Discs from each variant (with area 1,77 cm²) were placed in buffer media with pH 2,0, 3,0, 4,0, 5,0, 6,0, 7,0, 8,0 and 9,0. The

changes were recorded at every hour as a percentage alteration of the area. The results are presented as the mean of three replicates.

Statistical analysis. Results were analysed by statistical program Minitab 15. One-Way ANOVA and Tukey Test were used to determine statistically significant differences ($p < 0.05$).

RESULTS AND DISCUSSION

Analysis of the source pectin

The quantitative analyses of samples from the source pectin product showed content of pure pectin - 71,50%. As it is known the offered commercial products besides pure pectin also contain neutral sugars and other ballast substances. The content of the galacturonic acid was $753,0 \pm 14,76$ mg/g, and of the methoxy groups - $68,50 \pm 2,36$ mg/g. From the obtained results was calculated the degree of esterification which for this pectin type is 56,90%. By DE the used pectin was determined as high methoxyl but also with presence of a certain percentage free carboxyl groups.

pH of the film forming solutions varies from 2,88 for the pectin up to 6,16 for the sodium alginate (AG). The different variants film forming solutions show pH values in the acid area: C₁AG-P - 4,25; C₂AG-P - 3,78 and C₃AG-P - 3,54.

Characteristics of the obtained films

Visually the films were homogeneous, uninterrupted, with no brittle areas and without air bubbles (Table 1). They were easily manageable and flexible. The color of the alginate films varies from transparent to lightly opaque. The films with high pectin content were with lightly yellowish nuance. The thickness of the obtained materials depends on the applied technology and on the drying conditions, and is an important parameter for determining of the values of the mechanical characteristics. The most popular method for producing of films with controlled thickness is the pouring of a constant quantity film forming mixture for a given area. We applied one and the same proportion for the different variants - 0,325 g FS/cm² as the obtained films were with thickness from 0,039 up to 0,058 mm.

In this investigation the calcium chloride was used as gel forming and cross-linking agent. The polyvalent cations such as Ca²⁺, play the role of bridges between the anion polymer chains of the alginate and build junction zones which form the hydrogel network. The calcium ions are preferentially linked with the G-blocks of the alginate molecule due to the specific conformation structure of the polyguluronic chains and the presence of well defined places for chelate links [15]. It is considered that the forming of gel for the alginates follows the so called "egg-box" model according to which the ion links are between the calcium ions and two parallel G-chains. Notwithstanding that new data from MNR spectroscopy and x-ray diffraction suppose a presence of a more complex three-dimensional structure, the simple dimeric "egg-box" model is still valid as it is principally correct and gives a good idea of the linking properties of the alginates.

For the pectins with DE around 50%, the jellying capacity depends on a majority of factors – concentration of the pectin, the dissolved sugars, pH value of the solution and the quantity of the present calcium or other polyvalent cation. Gel forming of pectin solution was observed at high calcium ions concentration, which is an indication for a certain interaction between the ions and the HM pectin [16]. This process is not specific in contrast to the "egg-box" mechanism for the alginates and the LM pectins and according to Fang et al. [4], is usually polyelectrolyte interaction. The solutions of the two polymers were mixed very well in all studied proportions. The adding of calcium ions in low concentration (1,0 mM) leads to an increase of the viscosity of the mixture at places without local gel forming. For composition C₂AG-P, where the pectin and the sodium alginate are in equal proportions, the obtained result for viscosity was 386 mPa.s (at speed gradient 27,2 s⁻¹) at the beginning and increased up to 1300 mPa.s after adding of Ca²⁺. In such a way an easier manipulation after water evaporation was achieved. The real cross-linking of the resulting film took place after immersion in 0,3M solution of calcium ions.

Film forming solution C₂AG-P was with pH-value 3,78 – higher than pKa of the pectin which

Table 1. Characteristics of films

Composition	Appearance	Thickness (mm)	Residual moisture (%)
AG	transparent, flexible	0,054	11,52
C ₁ AG-P	lightly opaque, flexible	0,050	12,82
C ₂ AG-P	lightly yellowish, flexible	0,048	14,10
C ₃ AG-P	lightly yellowish, flexible	0,043	14,20

implies the presence of uncharged and charged carboxyl groups. After adding of calcium ions electrostatic as well as unspecific hydrophobic interactions with the pectin and specific with the alginate by the “egg box” mechanism were realized.

The residual moisture in the end products depends on a number of factors like the type of the used polymer, the concentration of the plasticizer, the conditions of cross-linking and drying. The films with greater alginate content are with lower residual moisture (Table 1). Up to a given concentration the water contained in the film plays the role of a plasticizer and increases the film elasticity. Residual moisture under 10% deteriorates the mechanical properties of the films and above 20% it can cause substantial changes in the protective properties, it can increase the permeability of the gases (or of the aromatic components) and to induce a danger of bacterial contamination.

Mechanical properties

Important parameters of mechanical properties are: tensile strength, elongation and elastic deformations modulus. The ultimate tensile strength (TS) is an assessment of the mechanical resistance, owing to the cohesion forces between the polymer chains. The elongation at ultimate strength (E) is the maximum change in the length of the tested material before breaking, expressed in percentage towards the initial length of the not deformed sample. The elasticity modulus (Young’s modulus, YM) is expressed as a ratio of linear stress to the linear strain ($Y = \text{stress}/\text{strain}$). The slope of stress-strain curve resulting from these tests present the dependence between the elongation and the applied strength (Fig. 1 and 2). The summarized results from the tests for mechanical properties of AG and C₂AG-P are given in Table 2. The alginate films (AG) showed the following values: TS - 18,30 MPa; E - 78,14 %. These results differ from some data mentioned in literature [17,18,19], as the lower TS values and the higher E values can be explained with the differences in the alginate concentration,

the applied plasticizer and the cross-linking method. For the composite film C₂AG-P the TS value is 53,53 MPa, and E is 34,48%. Usually the increase of TS leads to a decrease of the percentage for E [18, 20]. The obtained values for Young’s modulus increase from 16,61 MPa for AG up to 202,84 MPa for C₂AG-P. The increased TS and YM values and the decreased E values for the composite films can be explained by the higher cross-linking density when combining two anion polysaccharides, which leads to the forming of a more dense network and compact structure of the film after solvent evaporation.

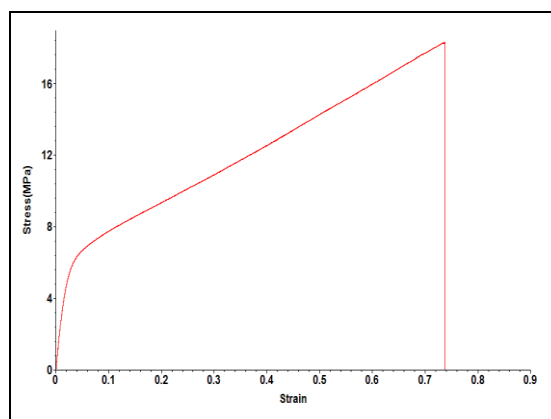


Fig. 1. The “stress-strain” curve for AG films

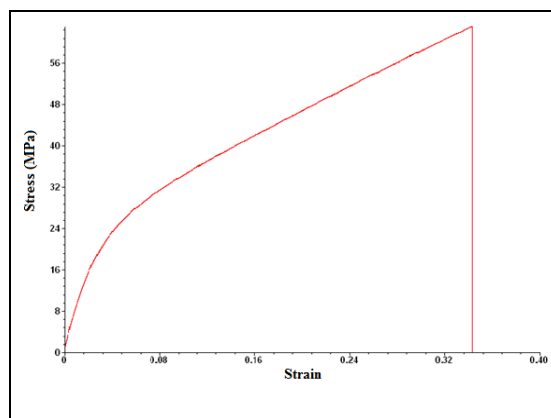


Fig.2. The “stress-strain” curve for C₂AG-P films

Table 2. Mechanical characteristics of AG and C₂AG-P

Mechanical characteristics	Composition					
	AG			C ₂ AG-P		
	Mean	SD	SE	Mean	SD	SE
Yield strength (MPa)	7,37	±0,54	±0,221	18.93	±3.84	±1.57
Ultimate tensile strength (MPa)	18,30	±0,68	±0,276	53.53	±4.25	±1.74
Young’s modulus (MPa)	16,61	±0,78	±0,319	202.84	±21.08	±8.60
Elongation at Ultimate strength (%)	78,14	±6,12	±2,50	34.48	±4.32	±1.76
Toughness (J/mm ³)	7,503	±0,797	±0,325	9.229	±3.143	±1.283
Energy at Yield point (J/mm ³)	0,296	±0,041	±0,017	0.324	±0.119	±0.048

In-vitro dissolution tests

On Figures 3, 4 and 5 are presented the results for the solubility of the films in simulated gastric and intestinal juice, prepared with different buffer media. These tests give a possibility to follows up the disintegration profile when taken with food. In a simulated gastric juice the pure alginate films kept their form and wholeness during the period of investigation, and for the composite film C₂AG-P a minimum disintegration was observed (10%). Much higher level of disintegration was observed when placing the samples in simulated intestinal juice, prepared with phosphate buffer (Fig. 3 and 4).

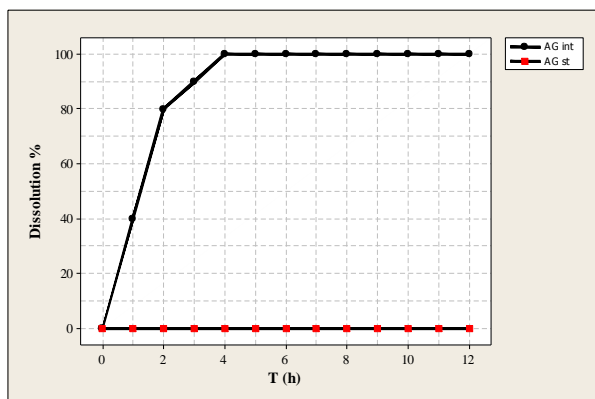


Fig. 3. Dissolution of alginate films in simulated gastric (AG st) and intestinal (AG int) juice

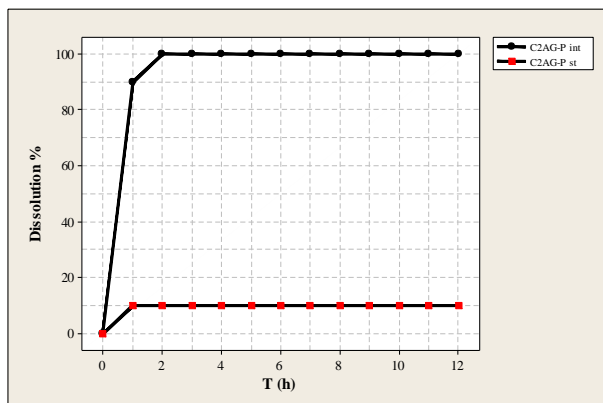


Fig. 4. Dissolution of composite films in simulated gastric (C₂AG-P st) and intestinal (C₂AG-P int) juice

For AG the changes begin yet at the first hour (40%) and reach up to 100% at the fourth hour. For the composite films an even faster disintegration was observed which reached 100% at the second hour. The experiment with simulated intestinal juice prepared with Tris buffer showed different results (Fig. 5). In this case the alginate and composite films were with a lower disintegration level: AG reached 70% disintegration at the fifth hour, and C₂AG-P – up to 80% at the fourth hour.

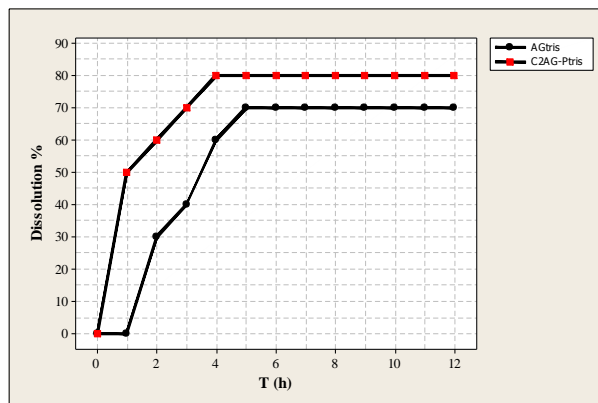


Fig. 5. Dissolution of alginate film (AG tris) and composite film (C₂AG-P tris) in simulated intestinal juice with Tris- buffer

The investigated polysaccharide films kept almost completely their wholeness at the low pH of the simulated gastric juice. When pH of the medium is under pKa of the respective uronic acids, the penetration of the water molecules, the swelling and the dissolving of the film were hampered. In the case of artificial intestinal juice the solubility was much higher, which is related not so much to the activity of the enzyme complex but to the pH of the medium. The buffer salts have an effect as well. The faster dissolving in the phosphate buffer is owing to the extraction of the calcium from alginate-Ca²⁺/pectin-Ca²⁺ complexes. When using a Tris-buffer the disintegration of both film types was slower and till the end of the period full dissolving was not reached.

These observations were also confirmed by the carried out tests for disintegration depending on the pH of the medium (Fig. 6 and 7).

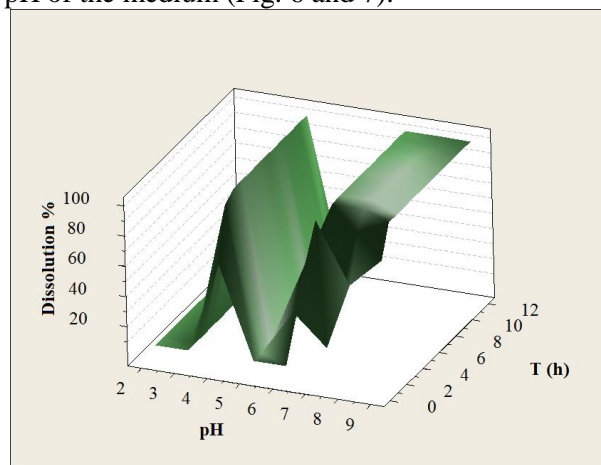


Fig.6. Dissolution of alginate film (AG) for different pH

The AG samples, placed in solutions with pH 2,0, 3,0 and 5,0 preserved their wholeness till the 12th hour. In a solution with pH 6,0 the disintegration of the film reached 60% at the 8th hour. At pH 4,0, 7,0, 8,0 and 9,0, the alginate film

was completely dissolved for a period from 1 to 3 hours. The results for C₂AG-P showed a different disintegration level compared to the pure alginate films. Complete dissolving of the samples was observed for solutions with pH 4,0, 5,0, 6,0, 7,0, 8,0 and 9,0. For the films placed in solutions with pH 2,0 and pH 3,0 at the end of the period the disintegration reached up to 20% and 75%, respectively.

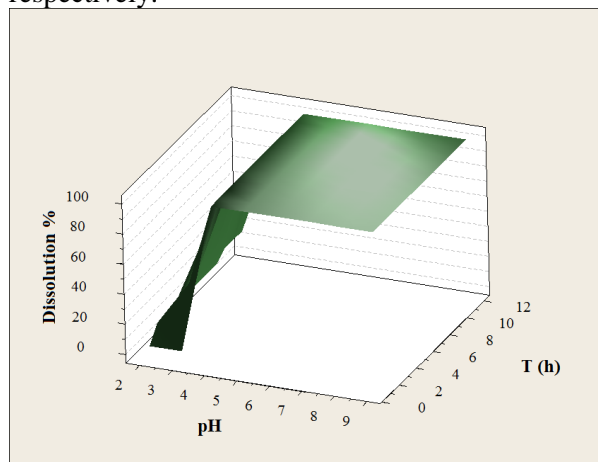


Fig.7. Dissolution of composite film (C₂AG-P) for different pH.

CONCLUSION:

By combining of sodium alginate and high methoxyl pectin were obtained well formed, homogeneous and semi-transparent films. The adding of pectin to the alginate film forming solution in proportion 1:1 improves considerably the film mechanical properties. The alginate and alginate-pectin films show no or very slow dissolution at low pH, including in simulated gastric juice. A difference is observed in the behavior of the two film types at pH values above 4,0, as the composite films show faster and full disintegration in the medium.

Due to the good mechanical characteristics and the selective solubility depending on pH, the composite alginate-pectin films are appropriate as material for encapsulation of biologically active substances or microorganisms, with low level of activity or survival, under low pH conditions.

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ПРИГОТВЯНЕ И ОХАРАКТЕРИЗИРАНЕ НА КОМПОЗИТНИ ХИДРОКОЛОИДНИ ФИЛМИ ОТ НАТРИЕВ АЛГИНАТ И МЕТОКСИЛИРАН ПЕКТИН

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(Резюме)

Разработена е технология за получаване на композитен биоразградим филм на основата на натриев алгинат и ябълков високометоксилиран (НМ) пектин. Установени са добри механични характеристики при използване на двата полизахарида в съотношение 1:1. За тях крайната якост на опън (TS) е 53.53 МПа, удължението при крайната якост (E) е 34.48%, модулът на Янг (YM) – 202.84 МПа. Композитният филм показва много слаба разтворимост при ниско рН, включително в симулиран стомашен сок. При стойности на рН над 4.0 алгинатните филми показват по-бавно, а композитните филми – по-бързо и пълно разпадане в средата. Благодарение на добрите механични характеристики и селективната разтворимост в зависимост от рН, композитните филми алгинат – НМ-пектин са подходящи като материал за капсуловане на биологично активни вещества или микроорганизми с ниска активност или оцеляващи при условия на ниско рН.