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Ethapolan: A New Microbial Exopolysaccharide for Oil Industry

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Ethapolan, a new high-viscous exopolysaccharide, was obtained in the course of microbial synthesis. Its chemical content and some characteristics of its solutions are explored in the present study. By its structure ethapolan may be considered a polysaccharide of xanthan type. More definitely, as compared to xanthan, the emulsifying efficiency and hydrophobic nature of ethapolan may be attributed to the presence of the fatty acids residues and to 6-desoxysaccharide—rhamnose residues. It was found that a few factors impact the increasing viscosity of ethapolan solutions: first, the presence of the cations; second, low shearing rates; and, finally, low pH values. Ethapolan is resistant to heating. On this basis, it may be concluded that ethapolan appears to be a universal and quite competitive microbial exopolysaccharide for the oil industry.

Introduction

Due to the current oil deficit, microbiological methods of oil extraction should draw our special attention. The use of water-soluble biopolymers or microbial exopolysaccharides is among most beneficial microbiological methods of oil extraction.

Microbial exopolysaccharide is used to increase the viscosity of the water pumped into the oil well and also to block up high-penetrating strata. It allows to control water and oil circulation in the pipe.[1] In the present study the following physicochemical characteristics are used to prove the eligibility of the microbial exopolysaccharide for a more effective oil-extracting process: first, the high viscosity of the low-concentrated solutions; second, sheer correlation between the viscosity of the solution and the shearing rate; third, the stability of the solutions—salts interaction at wide pH and temperature ranges; and, finally, its (exopolysaccharide's) resistance to oxidation and mechanical destruction.

Microbial exopolysaccharides are subjected to biological destruction. That is why application of the exopolysaccharides appears to be ecologically proven (ecologically safe method). The biocides are used to protect exopolysaccharides from biodestruction. If nothing else, formaldehyde is significantly efficient for the xanthan and scleroglucan solutions.[2] Likewise, some other biocides are used to prevent exopolysaccharides from destruction.[3]

Microbial exopolysaccharides have some advantages over poly(acrylamide), the polymer widely used in oil extracting. Poly(acrylamide) has some substantial drawbacks: oxidation, mechanical destruction, and coagulation in poly(acrylamide)—salts interaction, to name just a few. Moreover, poly(acrylamide) is resistant enough to biological destruction, so its wide use in oil extraction, leads to environmental pollution.

Currently, microbial exopolysaccharides, xanthan (producer *Xanthomonas campestris),*[4] scleroglucan (producer *Sclerotium rolfsii, s. glucanicum*),[5] and emulsan (producer *Acinetobacter calcoaceticus*)[6] are thought to be most beneficial/attractive for the oil-extraction process.

The primary goal of the present study was to explore the physicochemical characteristics of ethapolan, a new microbial exopolysaccharide for the oil industry.

Method

Ethapolan was synthesized from *Acinetobacter sp.*, which in turn was isolated from the active sludge at Nadvomyansky oil-processing plant sewage works (Ukraine). Codama, a mineral medium,[7] which contained ethanol (1% v/v) as a carbon source, was used for bacteria cultivation. Periodic cultivating was performed using fermenter AK-10 (4 L working volume). The culture in stationary phase of growth, developed in the flasks in the shaker, was taken as an inoculum. Preliminary air supply was 0.4 L/L of medium per minute, and the stirring rate was 150-200 min^{-1}. While the viscosity of the cultural fluid increased, the air supply was increased to 2 L per L of medium per minute, and mixer revolutions were 750 min^{-1}. During the incubation process the concentration of the dissolved oxygen was maintained at 20-40% of saturation.

Time of incubation was 36 h, at 29 + 1 °C, pH 6.8-7.0.

Ethapolan was isolated from the cultural fluid by ethanol sedimentation after the bacterial cells had been removed and dialysis had been performed.

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Carbohydrate content in exopolysaccharide was detected by phenol-sulfuric method;[8] protein content was detected after the Lowry method[9] and Bredford method;[10] to detect the overall content of inorganic components in the exopolysaccharide, the dry ashing method was used.[11]

Uronic acids were detected after Disch6;[12] pyruvic acid was detected as described;[13] to detect fatty acids weight method was used after ethapolan had been desacylated and fatty acids had been extracted by hexane.

Fatty acids qualitative test was performed using gas—liquid chromatography method (Pye Unicam Series-105). To detect neutral monosaccharides and uronic acids contained in exopolysaccharide of *Acinetobacter sp.*, the samples were hydrolyzed in the soldered ampules with 2 N trifluoroacetic acid for 2.5 h at 121 °C.

Neutral monosaccharides and uronic acids contents were analyzed by performing carbohydrate analyses using carbohydrate analyzer Biotronic LC-2000 (column 0.38 × 12.5 cm, with Dionex tar A × 8—11) in the 0.5 M sodium borate buffer (pH 8.0) at 60 °C and in the 0.04 M phosphate buffer (pH 2.4) at 70 °C, respectively. The neutral monosaccharides and uronic acids contents were detected by copper 2,2'-bicinchoni- nate treatment of the sample at 570 nm. ^{13}C NMR spectra of exopolysaccharide 1% solution in D_2O were monitored using Bruker Physik M-250 B spectrophotometer at 90 °C, taking dimethyl sulfoxide as an inner standard. IR spectra were monitored using UR-10 in tablets with KBr. Molecular mass was analyzed by the gel chromatography method (4B Sepharose column 70 × 0.9 cm), as well as by sedimentation and diffusion (MOM-3170v centrifuge), and, finally, analytical gradient centrifugation was performed to analyze the molecular mass method which was developed in our laboratory.[14]

The kinematic viscosity of the exopolysaccharides solutions was measured using Ostwald capillary viscosimeter, while the dynamic viscosity of the exopolysaccharides solutions was measured using Reomat-108 rotating viscosimeter.

Results and Discussion

1. **Chemical Composition.** Exopolysaccharide (ethapolan) is an acidic heteropolysaccharide, the molecular mass of which is 8×10^5 to 2×10^6 and higher. It should be noted, however, that the fractions of molecular mass over 2×10^6 dominate. It was detected that ethapolan contains (in %) carbohydrates, 40-45; uronic acids, 13.5—17.5; fatty acids, 1.5—6.0; pyruvic acid, 2.5—5.0; mineral compounds, 20-30. The humidity of the sample was about 10%.

Protein was absent or was not detected, a fact proved by both the negative Lowry and Bredford reactions and ^{13}C NMR spectrography data.

Neutral monosaccharides such as rhamnose, mannose,

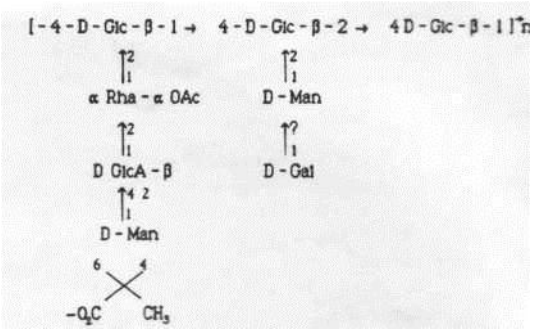

Figure 1. Possible structure of the repeated ethapolan link.

A fatty acids mixture was obtained in the course of ethapolan alkaline treatment. It appeared to comprise lauric, palmic/palmitic, palmitoleic, stearic, and oleic/ oleinic acids mainly (in ratio 10:29:12:7:20, respectively).

Ethapolan celit treatment did not impact fatty acids content in the sample; it did not impact the fatty acids ratio either. These results make us assume that fatty acids of ethapolan are of exogenous nature. In the ethapolan IR spectrum, the 1725 cm^{-1} absorbtion band is present, the band inherent to complex etheric bonds. However, in the IR spectrum of the modified sample, after 0.1 M NaOH treatment (when the fatty acids residues are cleaved), the 1725 cm^{-1} absorbtion band is absent. Possibly, monosaccharide residues are esterified by the fatty acids in ethapolan case.

Taking into consideration ^{13}C NMR spectrography data and the results of chemical analyses, it may be claimed that the principal chain of the *Acinetobacter sp.* polysaccharide is nothing but *β*-1,4-glucose residues, where two of every three glucose residues are bound to the side chains (Figure 1).

Thus, ethapolan appears to be a polysaccharide of xanthan type. As compared to xanthan, the presence of the "spare" side chain in the repeated link gives the additional confomatonary mobility to the molecule. At the same time it results in the increased stiffness of the ethapolan principal chain.

As a consequence, the viscosity of ethapolan solutions is higher than the viscosity of xanthan ones. The reason for more definite, as compared to xanthan, emulsifying efficiency and hydrophobic nature of ethapolan is the presence of the fatty acids residues and 6-deoxysaccha- ride, notably rhamnose residue.

2. **Characteristics of the Ethapolan Solutions.** The kinematic viscosity of 0.1% ethapolan solution is 5.5—10.0 mm^2/s (it depends on producer cultivation conditions and on the methods of isolation of ethapolan). It should be mentioned that the 0.1% Sigma xanthan solution's viscosity is 3.6—4.0 mm^2/s. Ethapolan solution is a steady pseudoplastic liquid, the viscosity of which decreases in a few seconds after the shearing stress is gone.

The data (see Figure 2) show the sheer correlation between ethapolan viscosity and the shearing rate. In the low shearing rates area (0.1—1.0 s^{-1}) ethapolan solution has Newtonian liquid characteristics. Pseudoplasticity of the flow becomes apparent at the shearing rate exceeding 1.0 s^{-1}. The changes in the viscosity rates (increasing), detected in low-concentration ethapolan solutions, were significant. For this reason, ethapolan as an oil-displacing agent draws our special interest.

In order to compare the ethapolan solution's rheological characteristics with those of Sigma xanthan

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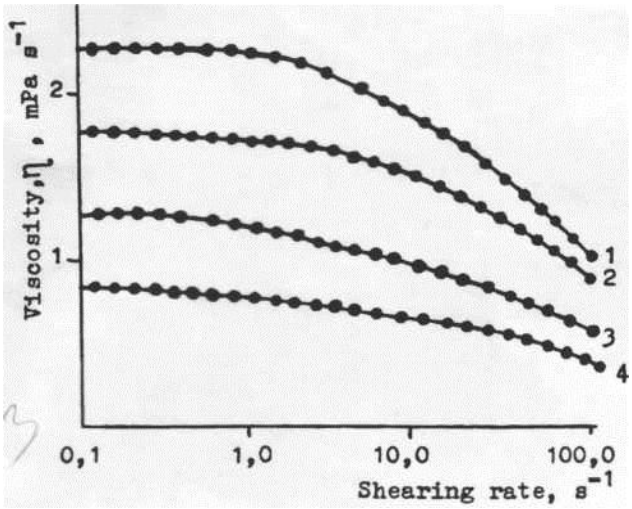

Figure 2. Correlation between the dynamic viscosity (η) of ethapolan solutions and the shearing rate. Concentrations of the solutions (%): (1) 0.175; (2) 0.0161; (3) 0.08; (4) 0.0063.

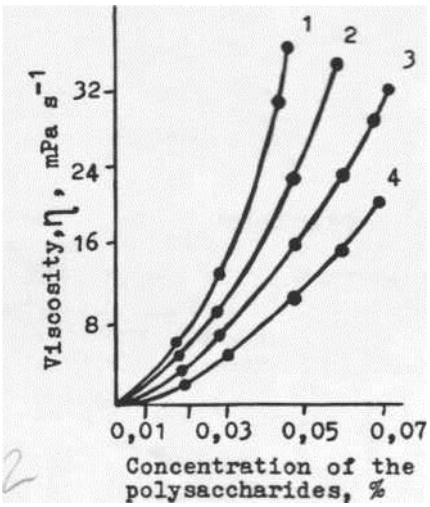

Figure 3. (1, 2) Correlation between dynamic viscosity *(η)* and concentration of ethapolan solutions at different shearing rates. (3, 4) Correlation between dynamic viscosity (η) and concentration of xanthan solutions at different shearing rates. (1, 3) shearing rate 1 s^{-1}; (2, 4) shearing rate 10 s^{-1}.

solution, the viscosity changes in ethapolan solutions were detected at different concentrations and at different shearing rates (Figure 3). Interestingly, no significant difference between ethapolan and xanthan solutions has been discovered at low concentration. However, when the concentration of the solutions was increased up to 0.05%, the viscosity of ethapolan solution was more than twice higher than that of the xanthan one. It is noticeable that the viscosity of ethapolan solution increased when the sample had been heated to 120 °C and then cooled rapidly (Figure 4). On the contrary, the viscosity of the xanthan samples after the same treatment came down slightly (Figure 4).

The capacity to be structuralized by one- and twovalent cations is one of the basic ethapolan characteristics (the same holds for the cations present in strata water). In the presence of 0.005 mol K^+ and Na^+ the viscosity of 0.1% ethapolan solutions decreases slightly; however, there is a tendency for the ethapolan solution's viscosity to increase, while the cation's concentration is being increased (Figure 5). Twovalent cations (Ca^{2+}, Mg^{2+}) cause the increasing of the ethapolan solutions

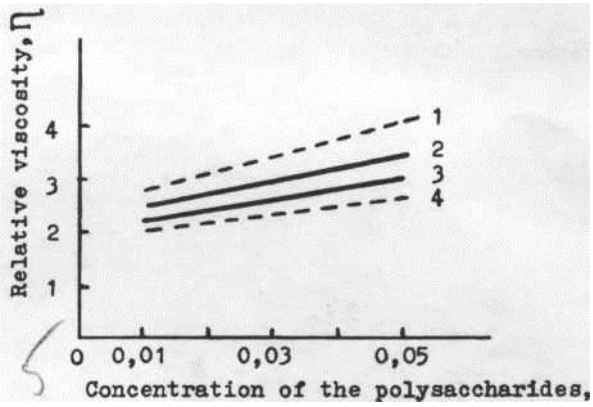

Figure 4. (1, 2) Effect of heat treatment on the viscosity (η relative) of ethapolan solutions. (3, 4**)** Effect of heat treatment on the viscosity (η relative) of Sigma xanthan solutions. (2, 3) Before heat treatment; (1, 4**)** after heat treatment.

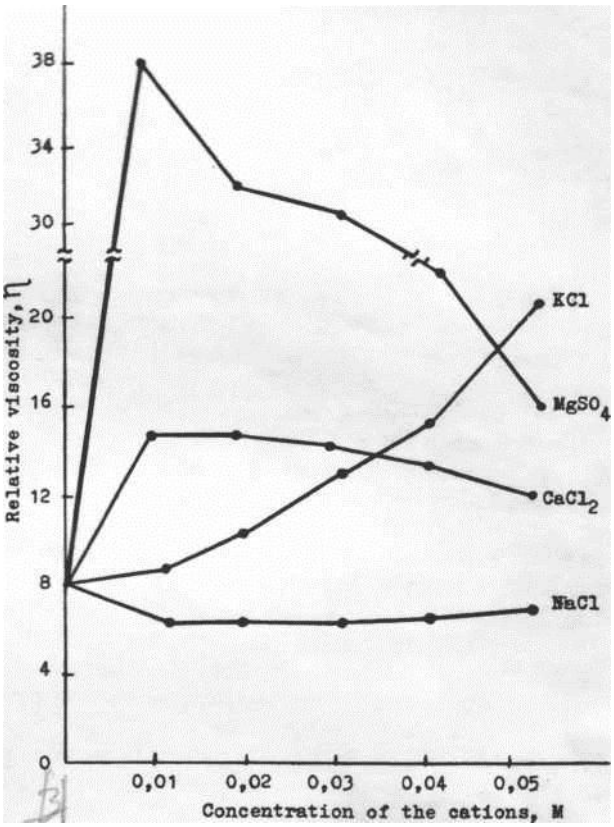

Figure 5. Effect of cations on the visocisty (η) of **0.1%** ethapolan solutions.

at 0.005 mole concentration, while at higher cations concentration the viscosity of the solutions decreases (Figure 5).

As in xanthan solutions, the viscosity of ethapolan solutions decreases slightly on heating, although, heating to 80 °C showed no effect on the cations structur- alizing capacity of the samples (Table 1). The results enable to use ethapolan in the mineralized solutions, as well as at higher temperatures. The presence of Al^{3+} and of Cu^{2+} causes ethapolan sedimentation. However, the sediment, obtained after ethapolan CuSC4 treatment (0.0015-0.003 mole) converts to a solution after glycine is added (0.007—0.015 mole). The significant increase in the solution viscosity occurs in the course of this treatment (Table 2). Thus, the 0.1% ethapolan solution viscosity in the Cu^{2+}—glycine system is com-

Table 1. Effect of Temperature on the Viscosity of 1% Ethapolan Solutions at Different Concentrations of Salts

salt	concn of salts (μ)	viscosity η rel to temp 20 °C	30 °C	50 °C	80 °C
no salt added	0.00	8.0	7.5	5.0	2.8
KCl	0.05	20.0	13.0	8.5	3.5
	0.10	24.0	14.0	9.0	3.75
	0.20	25.2	14.5	10.0	5.0
NaCl	0.05	7.0	5.8	4.4	3.0
	0.10	8.2	6.4	5.6	3.2
	0.20	10.4	8.8	6.6	3.8
$MgSO_4$	0.05	16.0	11.5	8.5	6.0
	0.10	14.0	11.0	7.5	5.5
	0.20	12.0	10.5	7.0	5.0
$CaCl_2$	0.05	13.4	10.6	7.2	3.6
	0.10	12.6	9.4	6.4	3.4
	0.20	10.8	9.0	6.2	3.4

Table 2. Dynamic Viscosity of Ethapolan Solutions, Pa s^{-1}, 20 °C

shearing rate	0.1% ethapolan solution Cu^{2+}, glycine	0.1% ethapolan solution in 25 days	1% ethapolan solution	1% xanthan solution
17.7	1.080	0.987	1.510	1.330
27.2	0.809	0.672	1.060	0.916
41.7	0.561	0.439	0.738	0.628
64.0	0.397	0.308	0.552	0.440
152.0	0.197	0.146	0.313	0.226
233.0	0.138	0.100	0.236	0.166
355.0	0.094	0.068	0.185	0.117

parable to the 1% ethapolan and xanthan water solutions. It is worth noting that the Cu^{2+} and glycine treatment extends the expiry date of ethapolan solutions (Table 2). Besides, ethapolan is more resistant to acids, unlike other polysaccharide solutions. As has been indicated, when pH of the sample was decreased from 7.0 to 2.6 the viscosity of the solution rose rapidly (Figure 6). Ethapolan was almost precipitated in the course of progressing acidification of the sample (pH up to 1.3). When constantly heated, the precipitate converted slowly to the solution. Thus, in 6 h only *88%* of the initial substance was left in 2 N H_2SO_4 at 100 °C. The sample's capacity to increase the viscosity at acidification (pH up to 2.6) makes ethapolan solutions very attractive as viscosifying agents in the pipes/ collectors with acidic water strata.

3. **The Use of the Ethapolan Solutions.** The gelforming compositions based on ethapolan were developed to make the exhausted oil wells at one of the Ukrainian oilfields waterproof. The ethapolan treatment of the oil wells caused a gradual increase in oil recovery throughout a year after the treatment, although the experiment is being carrying out currently. Ethapolan used for pumping was in the state of cultural fluid, obtained after producer fermentation. The cell- containing cultural fluid was subjected to plasmolysis and the biocide was added (notably formaldehyde 0.2%).

A mobile pilot plant was used to synthesize ethapolan on the spot. As a consequence, transportation and storage costs were minimized. Besides, there was no need for a dry product and its further dissolution.

Since ethapolan has a lipophilic component, fatty acids residues, it is capable of stabilizing water emulsions with oil and other hydrocarbons. Ethapolan then is quite attractive as an emulsifier to get rid of the oil residues in oil tankers, tanks, pipelines; ethapolan could be also used as a fuel additive.

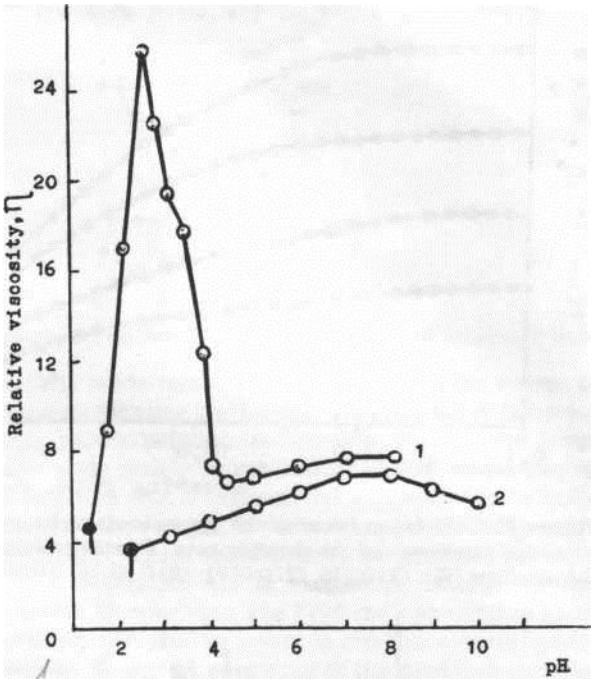

Figure 6. Correlation between the viscosity (η relative) of 0.1% ethapolan solutions and pH: (1) native/initial ethapolan; 92) deacylated ethapolan; (•) points of solution—precipitate (phase) conversion.

Ethapolan has some substantial advantages over other microbial exopolysaccharides used in oil-extracting process:

1. Its solutions have higher viscosity.
2. Due to the fact that the viscosity of the ethapolan solution increases in the presence of Cu^{2+} and glycine, ethapolan consumption comes down considerably (10 times as compared to xanthan); it also results in the stabilization of ethapolan solutions, extending the expiry date of the solutions.
3. The presence of one- and twovalent cations in concentrations inherent to the water strata makes the viscosity of the ethapolan solutions increase; while under the same conditions the viscosity of most polysaccharides decreases.
4. The viscosity of ethapolan solutions increases considerably in the low shearing rates area.
5. Ethapolan solutions have characteristics inherent to pseudoplastic liquids in both deionized water and in the presence of salts.
6. Ethapolan is exceptionally resistant to acid media, unlike most other biopolymers, which are decomposed in 2—5 h under the same conditions.
7. Ethapolan is resistant to heating.

Given this, it may be concluded that ethapolan appears to be a universal and quite competitive microbial exopolysaccharide for the oil industry.

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