

# ELECTROCHEMICAL METHOD FOR OBTAINING WATER-SOLUBLE OLIGOMERS OF CHITIN.

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## Abstract

Obtaining of water-soluble oligomers of chitin that possess such valuable properties as anti-tumor and immunostimulating activity, ability to activate intestine microflora and to protect plants from pathogens, especially of those having fungi nature, is considered to be very important. The insolubility of chitin restricts its usage, as for chitosan – it is very expensive and often less efficient than chitin. The existing methods used to lower chitin molecular mass – biological and chemical ones are ecologically unsafe, expensive and bring to formation of monomers and dimers mostly.

Electrolytical treatment of low mineralized water solutions in electrolyzers results in formation of oxidizers, the type and concentration of which is regulated by the choice of electrolytes, amount of electric energy used, structural features of electrolyzers and control of media consumption. It is known about the use of electrolyses in cellulose processing.

We have proposed to obtain water soluble oligomers of chitin by means of its electrolytic treatment inside both membrane and non-membrane electrolyzers being effected by mild acting system of oxidizers that has special composition being formed in the result of low mineralized water media electrolyses and under the effect of preset electric field voltage. Obtaining of the system of oxidizers excluding halogenation is achieved by controlling the concentration and type of electrolytes, parameters of electrochemical treatment and use of halogen-free salts. The identity of the obtained water-soluble chitins and their high-molecular predecessors structures have been shown in the paper.

## Introduction

Obtaining of water-soluble oligomers of chitin that possess such valuable properties as anti-tumor and immunostimulating activity, ability to activate intestine microflora and to protect plants from pathogens especially of those having fungi nature is considered to be very important. The insolubility of chitin restricts its usage, as for chitosan – it is very expensive and often less efficient than chitin. The existing methods used to lower chitin molecular mass – biological and chemical ones are ecologically unsafe, expensive and bring to formation of monomers and dimers mostly. Electrolytical treatment of low mineralized water solutions in electrolyzers results in formation of oxidizers the type and concentration of which is regulated by the choice of electrolytes, amount of electric energy used, structural features of electrolyzers and control of media consumption. Electrochemical methods of treatment due to the combined effect of the products of electrolyses and electric field make it possible to obtain targeted products of high quality in mild conditions with low concentration of reagents. This method provides for a relative ease in regulating the technological parameters of the process, small size of equipment, high level of automation and mechanization.

The Giprotybflot Institute (St.Petersburg) has developed a method [1] for obtaining chitin, chitosan and other valuable products of electrochemical treatment of crustacean raw material in specially designed electrolyzers [2].

The purpose of the present work is to study prospects of obtaining oligomers of chitins by means of electrochemical treatment in low mineralized water solutions of electrolytes.

## Materials and methods

Chitinol was chosen as an object of study. Chitinol is a chitin (TU 9289-005-00472437098) obtained by means of electrochemical demineralization and deproteinization of shellfish, Gammarus Pulex (TU 15-01-473-87).

The rate of destruction has been estimated according to the following parameters: yield of a soluble component of chitooligosaccharides – CHOS (extracted according to the method described in [3]), rate of chitin mass changing -  $\Delta X$ , characteristic viscosity of the polymer solution in DMAA/LiCl in Ubbelode capillary viscosimeter. The estimation of the properties of anolytes used in the work has been carried out according to pH, redox potential of the medium (Eh), active oxygen concentration defined by the iodometric method.

Chitinol was treated inside the anode chamber of a specially designed membrane electrolyzer having a unique construction.

## Results and discussions

During the first stage of the study chitinol has been treated in acid medium with the use of (0.1 -0.1) N  $\text{Na}_2\text{SO}_4$  as a base electrolyte. It helps to accumulate sulfuric and persulfuric acids near the anode at the account of discharge of sulfate-ions, ozone and oxygen due to water disintegration and a large number of highly active radicals [4]. It means that acid hydrolysis and oxidation of raw material which, in turn, contributes to the breakage of glycoside links can take place simultaneously.

The table 1 contains the results of chitinol treatment in 0.5 N  $\text{Na}_2\text{SO}_4$ .

**Table 1. Influence of treatment time at the rate of chitinol destruction and yield of its water soluble fraction**

Treatment time, min	15	30	50	90
$\Delta X$ , %	12.4	35.4	33.2	34.9
CHOS, %	7/6	30.1	25.3	16.4

Thus, electrochemical treatment results in the destruction of a certain part of chitinol, like 35% in the present case. The rate of destruction is only slightly affected by the strength of current and concentration of  $\text{Na}_2\text{SO}_4$  solution. It was revealed that  $\text{Na}_2\text{SO}_4$  concentration influences the kinetics of material destruction as in case of 0.25 N  $\text{Na}_2\text{SO}_4$  time necessary to reach maximal rate of destruction makes 45 minutes, and in case of 1.0 N  $\text{Na}_2\text{SO}_4$  it needs 26 minutes. The yield of water soluble chitinol fraction having reached its maximal value – 30% starts to reduce.

As a comparison, when chitinol was treated with anolyte (without direct influence of electric field on material), the rate of destruction used to reach its maximal value (29%) in 24 hours, the yield of chitooligosaccharides being 15.9%.

Higher values of destruction rate at electrochemical treatment are explained by polarizing effect of electric field which facilitates interaction of chitinol with active components of the solution and considerably enhance the destruction of chitin. Slowed down kinetics of chitinol interaction with anolyte (in the absence of electric field) seems to result in the fact that in line with destruction active disintegration of chitooligosaccharides takes place.

It is known that treatment of polymers with alkali results in swelling and disordering of amorphous interfibrillar areas which may result in material destruction [5]. Besides, electrolyte ions can additionally affect material. It is known that ions of Sodium interacting with crystallite surfaces and amorphizing them reduce the rate of crystallinity and enhance the rate of disordered sections in its amorphous areas [6]. Thus ions of Sodium act as an anti-bonding agent. Joint influence on material rendered by electric field, oxidizers and radicals with high sorption properties, hydroxyl ions and ions of sodium can provide high destruction of chitin.

Similar to the first described experiment, electrochemical treatment of chitin in alkali medium with the following parameters  $\text{pH} = 12.000 \pm 10$ ,  $E_h = -(50 \pm 10)$  mV, continuous adding of NaOH to neutralize emerging on the anode protons similar results in destruction of only a certain part of chitin – 29%.

At the second stage of investigation electrochemical treatment of chitin was performed in (0.1 – 1.0) N solutions of NaOH in different pH spectra closely connected with the changes of electrochemical potential from –100 mV to +(162 – 1200) mV which corresponded to a single introducing of NaOH in the beginning of the process. The established dependence between the concentration of alkali and rate of destruction is given in the table 2.

**Table 2 – Influence of treatment time and NaOH concentration on  $\Delta X$**

NaOH concentration	Time of material electrochemical treatment, min	pH	$\Delta X$	CHOS, %
0.1 N NaOH	18	9.41	47.5	6.2
	50	-	-	-
0.5 N NaOH	18	12.76	20.1	19.0
	50	2.06	21.2	2.0

The results of the study helped to establish dependence of the rate of destruction from alkali concentration: the less concentration is the larger the rate of destruction, which might be stipulated by a strong influence of electric field polarizing effect on material at lowering electrolyte solution concentration caused by voltage rise in the process of electrolyses. As soon as the maximal number of oxidizers in each of the systems being measured by iodometric method is similar for all the cases – appr. 250 mg/l and practically does not depend on the concentration of NaOH solution and treatment time. The rate of destruction having reached its limit value does not increase any more. The maximal yield of chito oligosaccharides regardless of NaOH concentration makes 17%, further treatment of material leads to a decreased yield of chito oligosaccharides. Bringing of electrolyses process to lower pH values – 9 or less results in extremely intensive destruction of water soluble chitin oligomer fraction. It can be explained either by origination of new active destructors in line with pH reduction, or by acceleration of the destructive process and increase of oxidizers' destructive force at transition to neutral and, after that to acid area, or by reducing of resistance of obtained oligomers while passing into acid form. Thus for 0.5 N NaOH the yield of chito oligosaccharides makes 19% after 18 min treatment ( $\text{pH} = 12.76$ ), after 30 minutes ( $\text{pH} = 11.93$ ) – 17%, and after 40 minutes ( $\text{pH} = 2.36$ ) it lows down to 5%, that is why it is not rational to carry out electrolyses at neutral and acid pH values in the medium of this electrolyzer. At the same time, carrying out of the process in acid media  $\text{Na}_2\text{SO}_4$  (table 1) makes it possible to achieve much better results.

Lowering of solution electrical conductance caused by hydroxyl ions discharge on the anode leads to voltage increase and, correspondingly to serious loss of current. It is clearly expressed in experiments with the use of 0.1 N water solutions of NaOH. When concentrations of solutions increases (table 2) the time of electrolyses being characterized by stable voltage and, as a

consequence, minimal current losses makes it possible to reach maximal yield of polysaccharides, Thus electrochemical treatment of chitin in water solution of NaOH without additional introducing of electrolyte to support constant pH values and, as a consequence, constant electric conductivity of solutions permits not only to reach a satisfactory result but to reduce expenses on reagents.

Enhancing of current strength does not result in the chitooligosaccharides yield increase, there is a certain decrease of 10% when the strength of current is increased twice as much (yield of chitooligosaccharides is 20% instead of 30%) and it seems to happen due to the acceleration of chitooligosaccharides destruction reaction.

At the final stage of the study electrochemical treatment of chitin in  $\text{Na}_2\text{SO}_4\text{-NaOH}$  medium at single introducing of reagents in the beginning of the process has been carried out. Electrochemical treatment including transition from alkali to acid area, might permit to enhance speed and rate of material destruction and yield of polysaccharides. Introducing of  $\text{Na}_2\text{SO}_4$  will ensure voltage stability during electrolyses and will permit to carry out destruction at any possible pH values, including pH=7. This assumption is based on the results given in the tables 1 and 2.

The interaction between the time of chitin treatment and  $\text{Na}_2\text{SO}_4$  concentration with the rate of destruction and yield of water soluble fraction of chitin presented in the table 3.

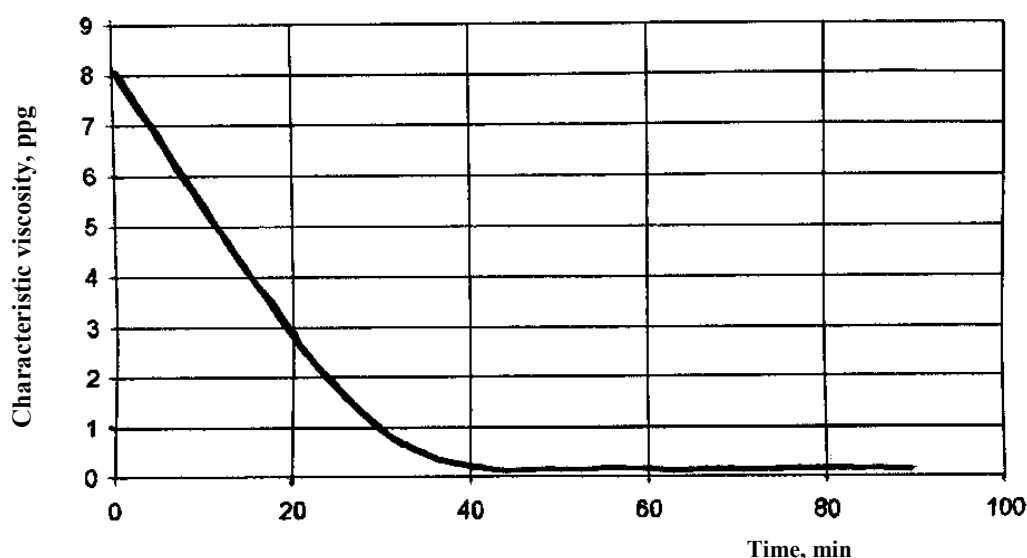
**Table 3 – Influence of treatment time and  $\text{Na}_2\text{SO}_4$  concentration at the chitin destruction rate and yield of water soluble fraction**

$\text{Na}_2\text{SO}_4$ concentration	Time of electrochemical treatment of material, min	$\Delta X$ , %	CHOS, %
0.10 N	10	38.2	32.4
	30	50.8	38.4
	60	58.4	21.6
0.50 N	10	64.6	42.3
	15	79.8	38.5
	60	35.4	15.3

The table data shows that the duration of treatment depends upon the  $\text{Na}_2\text{SO}_4$  concentration. The yield of chitooligosaccharides is of an extreme nature: having achieved its maximum (on the 30<sup>th</sup> minute of treatment in 0.5 N solution of  $\text{Na}_2\text{SO}_4$  and on the 10<sup>th</sup> minute in 0.1 N solution of  $\text{Na}_2\text{SO}_4$ ) chitooligosaccharides start to destruct. In solutions with 0.5 N concentration of  $\text{Na}_2\text{SO}_4$  and higher the speed and rate of destruction are high but the yield of chitooligosaccharides is not satisfactory. Optimization of conditions of the process:  $\text{Na}_2\text{SO}_4$  and NaOH concentration, time and electrochemical parameters of treatment ensured relatively high yield of chitopolisaccharides – 64.8%. Losses (difference between the chitin that went into solution and chitooligosaccharides extracted from the solution) evidently stipulated by the oxidizing destruction of the latter made 7%. Changing of chitin structure in the process of electrochemical treatment are confirmed by the results given in the fig.1, that show changing of viscosity while treating chitin in the mixture of  $\text{Na}_2\text{SO}_4$  and NaOH at the transition of pH values from alkali to acid area. Characteristic viscosity achieved by this method of treatment makes 0.4 ppg, which is 5 times as lower than characteristic viscosity of chitosan being obtained during treatment by sulfate in acid medium and three times as lower as in case of chitin obtained in the result of electrochemical treatment in alkali media. The character of the curve demonstrates the presence of limit viscosity of chitin.

The structure of the obtained chitooligosaccharides has been studied by IR-spectroscopy methods (with the use of Perkin Elmer 16 PC FT-IR in KBr pills, Peak Feak for Windows software package has been used to separate peaks on the curve) and potentiometry titration method, IR-spectroscopic images of chitooligosaccharide samples obtained in mild conditions (at low concentrations of  $\text{Na}_2\text{SO}_4$ ) demonstrate absence of structural changes except certain deacetylation material. Increasing of treatment time resulted in origination of oxidized carbon and increasing of amino groups number at the account of deacetylation. The use of more concentrated electrolytic solutions is undesirable as it may lead in addition to acetylation to a certain deamidation and oxidizing of carbon, which considerably changes the structure of chitooligosaccharide monomer links.

Another product obtained during electrochemical treatment, beside water soluble fraction of chitooligosaccharides, is activated non-soluble in water chitinel with new properties – enhanced lipid sorption ability in particular.



**Figure 1 :** Influence of time used for electrochemical treatment of chitinel at its characteristic viscosity

## Conclusion

Electrochemical method of obtaining water soluble oligomers of chitin that implies electrochemical treatment of high molecular chitinel in the anode chamber of electrolyzer with the use of direct electric current has been developed.

The effect of reducing molecular mass is achieved due to a complex effect rendered on raw material by low voltage electric field and oxidizers being synthesized on the anode during electrolyzing of water and hydroxyl ions at the background of pH values 12.0 – 1.0 changing in case of  $\text{Na}_2\text{SO}_4$  and  $\text{NaOH}$  electrolyzing. It is shown that such treatment of chitinel provides high rate of destruction and high yield of chitooligosaccharides. The maximal yield of chitooligosaccharides makes  $62.4 \pm 2\%$ .

In line with water soluble chitooligosaccharides non-soluble chitin with a changed structure and properties has been obtained. It has enhanced sorption ability which might be stipulated by reducing of its molecular mass.

The advantages of electrochemical destruction method are as follows: low concentration of oxidizers, absence of halogenation and possibility to control pH, quickness and environmental safety of the process.

It was found that by changing treatment time and  $\text{Na}_2\text{SO}_4$  concentration it is possible to regulate the structure and yield of the products obtained.

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