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Li ³ ЛИТИЙ	Be ⁴ БЕРИЛЛИЙ		
Na ¹¹ НАТРИЙ	Mg ¹² МАГНИЙ		
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29 Cu МЕДЬ	30 Zn ЦИНК		
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47 Ag СЕРЕБРО	48 Cd КАДМИЙ		
Cs ⁵⁵ ЦЕЗИЙ	Ba ⁵⁶ БАРИЙ		
79 Au ЗОЛОТО	80 Hg РТУТЬ		
Fr ⁸⁷ ФРАНЦИЙ	Ra ⁸⁸ РАДИЙ		

ВЛИЯНИЕ ЛИПАЗЫ НА МИЦЕЛЛООБРАЗУЮЩУЮ И СОЛЮБИЛИЗИРУЮЩУЮ СПОСОБНОСТЬ НЕИОНОГЕННЫХ ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ

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Биотехнология – одна из динамично развивающихся отраслей промышленности, которая нуждается в успешном интегрировании в уже имеющиеся технологии. Перспективным направлением является сочетание традиционной обработки целлюлозы поверхностно-активными веществами и ферментами для предотвращения проблемы смоляных затруднений. В настоящей работе представлены результаты исследования мицеллообразующей и солюбилизационной способностей отечественных неионогенных ПАВ (синтамида-5, синтанол ДС-10), ферментов липазы и их смесей. На основе коллоидно-химических характеристик подобраны оптимальные синергетические композиции исследуемых веществ. Установлено, что оптимальная добавка липазы до 30% не оказывает влияния на мицеллообразующие свойства поверхностно-активных веществ. Преобладание поверхностно-активного вещества во всех композициях снижает их стоимость. Наибольшее отклонение от аддитивных значений поверхностной активности наблюдается при смеси индивидуальных неионогенных поверхностно-активных веществ и липазы в соотношении 70:30. Однако в смесях обоих тензидов и липазы отношение экспериментальной поверхностной активности к теоретически рассчитанной менее единицы. По-видимому, гидрофобные участки смешанных агрегатов блокируют гидрофобные участки липазы тем самым предотвращая адсорбцию липазы на межфазной поверхности. Наибольшей солюбилизационной емкостью обладает синтанол ДС-10, обладающий более высоким гидрофобно-гидрофильным балансом, низкой критической концентрацией мицеллообразования, что приводит к образованию большого количества мицелл в растворе и увеличению суммарного объема гидрофобного ядра. Установлено, что количество солюбилизированной канифоли не зависит от концентрации липазы в растворе. Интенсивное растворение канифоли наблюдается в смеси синтанола ДС-10 и липазы, что предопределяет выбор таких систем для обессмоливания целлюлозных полуфабрикатов.

Ключевые слова: поверхностно-активные вещества, липаза, обессмоливание, синергизм в смесях амфифилов

IMPACT OF LIPASE ON MICELLE FORMATION AND SOLUBILIZATION ABILITIES OF NON-IONIC SURFACTANTS

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Biotechnology is one of the fastest growing sector of scientific and applied activities of the humans, which needs to be successfully integrated into existing technologies. Such upcoming trend is the combination of conventional pulp treatment by surfactants and enzymatic processing in order to prevent pitch troubles in the pulp and paper mills. This article presents the research results of the abilities of non-ionic surfactants (sintamid-5, sintanol DS-10), enzyme (lipase) and their synergistic combinations to the micelle formation and solubilization. We chose the optimal synergistic compositions and investigated their colloid-chemical characteristics. There is no effect to the micelle formation ability of surfactants when addition of lipase is up to 30%. The largest deviation from the additive values of surface activity was observed for the mixture of individual non-ionic surfactant and lipase at the ratio of 70:30. However, in the all mixtures of both surfactants and lipase the ratio of experimental surface activity to the theoretically calculated is less than one. It looks, that hydrophilic areas of mixed aggregates block hydrophobic areas of lipase thereby preventing adsorption of lipase at the interface. A predominance of the surfactant in the composition will reduce its cost. The maximum of solubilizing capacity has sintanol DS-10 due to its highest HLB and the lowest CMC that leads to more micelles amount in solution and higher total hydrocarbon volume. The pitch solubilization in lipase solutions does not depend on enzyme concentration. The high pitch dissolving in synergistic mixture of sintanol DS-10 and lipase is observed. It is predetermines the usage of such systems for cellulose deresination.

Keywords: surfactants, lipase, deresination, synergism in mixtures of amphiphiles

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INTRODUCTION

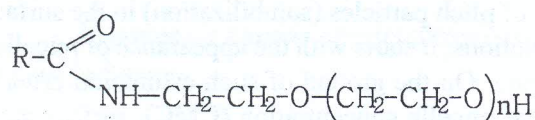
To solve a number of actual problems of pulp and paper industry (PPI) it is necessary to examine in details the processes on submicroscopic level. This will allow helping to intensify the process and making it cheaper. Pitch troubles are the old existing problems in PPI. They are generally evident as sticking of resin remaining in pulp after wood cooking on equipment and quality deterioration of finished product. This problem is particularly apparent when hardwood used for kraft process due to the predominance of fats in their parenchyma cells. Surfactants are conventionally used for deresination as well non-chemical methods

are widely used struggle with harmful pitch deposits [1]. The deresination process mainly is a colloidal dissolving of pitch particles (solubilization) in the surfactants solutions; it starts with the appearance of micelles in solution. On the ground of such estimation criteria as critical micelle concentration (CMC), surface activity and solubilizing capability it is possible to choose the ratio of surfactants in the mixtures, when the synergistic effect appears. The outstanding feature of surfactants as solubilizers is their flexibility to use solvent, this defines the wide area of their applications, as well allows creating the tolerant environment for activity of different biologically active supplements.

The main global ecological trend is to reduce the negative anthropogenic effect on the environment. This can be achieved by improvement of the existing processes with implementation of biotechnologies [2-4]. A particular area of biotechnological development in the PPI is the use of lipase enzymes against pitch troubles. Their certain advantage over surfactants is effective action and environmental safety [6]. The main constriction of lipase common usage is its high cost. So it is worth to investigate the joint usage of surfactants and lipase. But still there are no established reasons of mutual activation or inactivation of these substances. Limitation and inconsistency of the literature data does not allow to determine in full degree the interactions occurring in enzyme-amphiphilic multicomponent systems [2,6,7]. However, the combined application of amphiphilic substances and enzymes frequently leads to effective regulation of deresination process in target way.

EXPERIMENTAL TECHNIQUES

To study the colloid-chemical characteristics of deresination agents the following substances were chosen. Russian produced non-ionic surfactants Sintamid-5 (mixture of polyoxyethylated ethers of synthetic fatty acids monoethanolamides; is a translucent yellow liquid with the assay percentage at least 93%; highly soluble in soft and hard water and some organic solvents; not precipitating from a solutions of mineral acids and salts; water solutions become opaque in the presence of NaOH; pH of 1% water solution is 7-8; toxic to the water environment) and Sintanol DS-10 (mixture of polyoxyethylene glycol ethers of synthetic primary higher fatty alcohols; is a white or yellowish paste with the assay percentage 99%; very soluble in water; stable in solutions of mineral acids, alkalis and salts and also in hard water; pH of water solution is 6-9; has a negative impact on the environment) (Fig. 1) [8] and commercial enzyme Lipex 100L (Novozymes) obtained by submerged fermentation of genetically modified *Aspergillus*.



R = C₇-C₁₇; n = 5-9
Sintamid-5

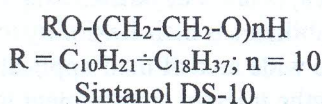


Рис. 1. Структурные формулы неионогенных ПАВ
Fig. 1. Chemical structures of non-ionic surfactants

Measurements of the surface tension were made by "Du Nouy method" [9,10]. The diameter of the platinum ring was 22 mm. There is no typical minimum for impurities on the surface tension isotherm so all objects of research were used without additional cleaning. The relative measurement error of the surface tension was 5%.

Amphiphile's solubilizing ability studies were performed in terms of the method described in [11,12]. Absorbance measurements of rosin centrifuged equilibrium solutions were carried out on the spectrophotometer SF-2000 at $\lambda = 298$ nm and path length of 1 cm. The content of solubilized rosin was determined by created calibration curves. The relative measurement error of spectrophotometric measurements was 3%. All research results were averaged between 3-4 replicates.

RESULTS AND DISCUSSION

Dependencies of surface tension versus aqueous solutions concentration of selected non-ionic surfactants and their mixtures were analyzed at the present work, CMCs were determined and surface activity G was defined. Critical micelle concentration is the most important colloid-chemical characteristic of the amphiphilic compounds determining their performance characteristics.

It is found that CMCs of Sintamid-5 and Sintanol DS-10 are 0.016 and 0.006 wt.% respectively. It has been established that Sintanol DS-10 (606 mJ·m/kg) has higher surface activity compared to Sintamid-5 (271 mJ·m/kg).

In practice surfactants are used as mixture of two or more components to provide synergistic or antagonistic effects [13]. This phenomenon can be characterized by the ratio of the maximum CMC deviation from the additive values to the additive value in the mixture composition which corresponding to this maximum deviation [14]. Scientific basis of the mechanism of mutual activation or inactivation in multicomponent mixtures of variable composition is not enough investigated. Therefore, there are difficulties with the deresination compositions creation which are prepared mainly empirically. Despite existing research on cellulose deresination by surfactants and their mixtures [1,15-18] there is no systemized data of resin reduction with the help of compositions surfactants and enzymes due to abundance of empirical data absence.

The ratio between experimental (exp) and theoretically calculated (add) values of critical micelle concentration and surface activity in the surfactants mixtures are explored to assess their interaction. The results are shown in table 1. It's apparent that selected mixtures have both antagonistic and synergistic effects

in comparison to additive values which are associated with the mixed micelles formation.

Таблица 1

Динамика неаддитивности в бинарных смесях неионогенных ПАВ

Table 1. Non-additivity dynamic in binary mixtures of non-ionic surfactants

Mixture Ratio of sintanol DS-10: sintamid-5, wt.%	$\frac{CMC_{add}}{CMC_{exp}}$	$\frac{G_{exp}}{G_{add}}$
10:90	2.27	1.93
20:80	1.79	1.55
30:70	2.60	2.09
40:60	2.60	2.03
50:50	2.04	1.66
60:40	5.26	0.48
30:70	6.00	4.94
20:80	4.00	3.49
10:90	4.38	3.96

Mutual activation in the mixture of sintamid-5 and sintanol DS-10 is foremost expressed at a ratio of 30:70. Its CMC is six times less than calculated additive value, while surface activity increasing in five times. Reduction of CMC defines a decrease of reagent's consumption. Concurrently economic effectiveness of surfactants application for cellulose deresination increases.

This paper presents the impact of lipase on surface and micelle forming properties of surfactants (Fig. 2,3) and theirs the most synergistic mixture (Table 2).

In view of the foregoing figures it is apparent that in the full range of the selected ratios CMC's synergistic reduction associated with the formation of mixed associates can be observed. But at low lipase content its effect on the micelle forming ability is minimal as distinguished from surface activity for which the maximum deviation from additive values appears in mixture of individual surfactant and lipase at the ratio of 70:30.

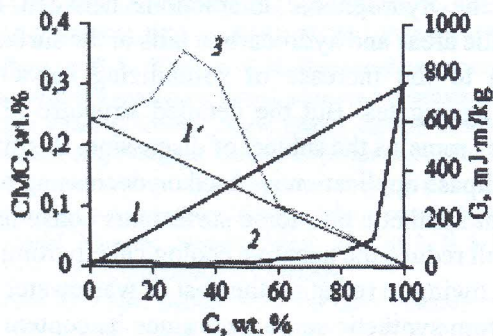


Рис. 2. Влияние добавки фермента липазы С на ККМ и G синтанола ДС-10. 1 – аддитивные значения G и ККМ; 2,3-экспериментальные значения ККМ и G соответственно

Fig. 2. Effect of the lipase addition on CMC and G of sintanol DS-10. 1 – additive values of G and CMC; 2, 3 – experimental values of CMC and G, respectively

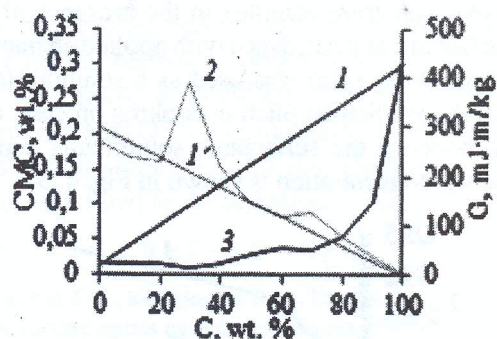


Рис. 3. Влияние добавки фермента липазы С на ККМ и G Синтамида-5. 1 – аддитивные значения ККМ и G; 2, 3-экспериментальные значения G и ККМ соответственно

Fig. 3. Effect of lipase addition on CMC and G of sintamid-5. 1 – additive values of CMC and G; 2, 3 – experimental values of G and CMC, respectively

However, the same cannot be said about the surface activity of the composition of synergistic binary mixtures of surfactants and lipase. The ratio of the experimental surface activity to its theoretically calculated additive value for all compositions of non-ionic surfactants mixture and lipase is less than unity (Table 2). This shows the competitive behavior pattern of mixed aggregates. Possibly that hydrophilic regions of mixed aggregates shield the lipase hydrophobic regions, thereby preventing the lipase adsorption process at the interface.

Таблица 2

Динамика неаддитивности в композиции синергетической смеси неионогенных ПАВ и липазы
Table 2. Non-additivity dynamic in composition of non-ionic surfactants synergistic mixture and lipase

Mixture Ratio of lipase:surfactants mixture, wt.%	$\frac{CMC_{add}}{CMC_{exp}}$	$\frac{G_{exp} \cdot 10^3}{G_{add}}$
10:90	3.14	149
20:80	8.62	232
30:70	18.58	381
40:60	12.73	228
50:50	14.36	250
60:40	18.06	335
30:70	12.68	263
20:80	9.24	267
10:90	4.50	227

The optimal amount of enzyme is 30% at a predominant content of non-ionic surfactants in all mixtures. Surfactant predominance in the composition will reduce its cost. The structure of the amphiphilic molecules determines the characteristics of supramolecular structures formed by them [5], so special attention is paid to the study of solubilizing capacity of amphiphilic substances. Solubilization of the resin particles, which are used as a substrate for the lipase action, will depend directly on the nature of the interaction of

the enzyme-substrate complex in the presence of non-ionic surfactant. In accordance with applied character of the present work, rosin was used as a solubilize and model of the emulsified pitch in cooking process. Relationship between the surfactants solubilizing capacity and solution concentration is shown in Fig. 4, 5.

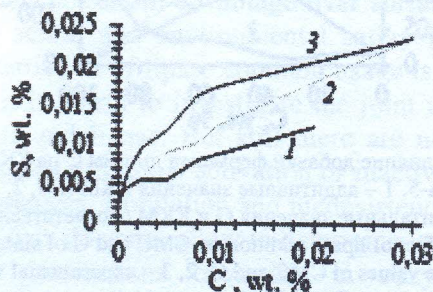


Рис. 4. Зависимость равновесного количества солюбилизированной канифоли S от концентрации растворов C . 1 – синта-мид-5; 2 – смесь синтанол ДС-10-синта-мид-5 (70:30); 3 – синтанол ДС-10

Fig. 4. Relationship between the equilibrium amount of solubilized rosin S and solution concentration C . 1 – sintamid-5; 2 – mixture of sintanol DS-10 and sintamid -5 (70:30); 3 – sintanol DS -10

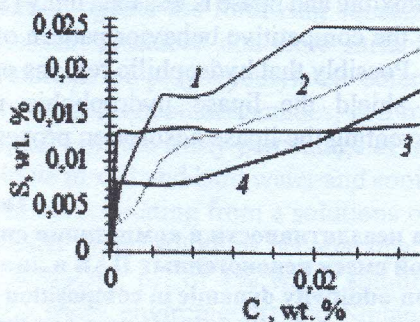


Рис. 5. Зависимость равновесного количества солюбилизированной канифоли S от концентрации растворов C . 1 – смесь синтанол ДС-10-липаза (70:30); 2 – смесь синтанол ДС-10-синта-мид-5+липаза (70:30); 3 – липаза; 4 – смесь синта-мид-5+липаза (70:30)

Fig. 5. Relationship between the equilibrium amount of solubilized rosin S and solution concentration C . 1 – mixture of sintanol DS-10 and lipase (70:30); 2 – mixture of sintanol DS-10- sintamid -5 and lipase (70:30); 3 – lipase; 4 – mixture of sintamid -5 and lipase (70:30)

As can be seen from Fig. 4 rosin solubilization appears at CMC of non-ionic surfactants and then abruptly increases which is typical for the phenomenon of solubilization. Induced water colloidal dissolving of organic substances is achieved in the presence of a sufficient amount of micelles (associates). An increase of the surfactants solubilizing ability is noticed with increasing of its concentration due to quality changes of solution colloidal structure (micelles rearrangement, micellar weight, and aggregation numbers). The larger is the surfactants micelle concentra-

tion the bigger amount of substance can be dissolved. This is expressed in abrupt increasing of the solubilized rosin amount after CMC. This should be considered during application of these substances.

It has been found that the rosin net water solubility in the absence of amphiphiles is 0.005%. The high values of solubilized rosin concentrations in surfactants molecular solution show rosin dispergating due to Reh binder effect [19].

Hydrophilic lipophilic balance (HLB) of the non-ionic surfactants was calculated by the Davis formula [20,21]. It is found that the HLB decreases in the sequence of sintanol DS-10>non-ionic surfactants mixture>sintamid-5 (18.3, 16.7, 13.2 respectively). Sintanol DS-10 possessing less CMC and high HLB has more micelles in solution versus others. This promotes the increasing of the hydrophobic core effective volume. Solubilization study results showed that in a mixture of non-ionic surfactants compared to the individual substances desirable synergism of mutual influence are not observed. This is probably due to the complicated organization of mixed micelles and steric hindrance when solubilize embedding them. While increasing the concentration of lipase the increase of solubilized rosin content is not observed versus the normal process of solubilization by surfactants (Fig. 5, curve 3). A constant value has been observed after a sharp increase of the solubilized rosin concentration. Apparently, there are difficulties with access to lipase hydrophobic sites responsible for the induced colloidal dissolution with increasing protein content. The most pronounced in the composition of sintanol DS-10 and lipase synergistic effect (Fig. 5, curve 1) should be noted. Presumably, sintanol DS-10 has a greater affinity with the lipase lipophilic areas due to surfactant's large volume of micelle hydrocarbon core. It determines the hydrophobic interactions between lipase lipophilic areas and hydrocarbon tails of the surfactants leading to the increase of solubilizing capacity of mixed aggregates. But the detailed structure of such complex remains the subject of discussion. Positive effect of lipase application is based on decreasing of bio-resistant synthetic non-ionic surfactants consumption. This will reduce the negative ecological environmental impact owing to reducing the cost of wastewater treatment from synthetic surfactants since its content there will be 30% less.

Totality of the submitted data allows evaluating the priority of synergistic compositions of synthetic surfactants and enzymes application in order to reduce total pitch content in wood pulp.

CONCLUSION

It was found that synergistic effect of micellization is disclosed when investigated surfactants interacting. It has been established that in micellar solutions rosin solubilization occurs owing to the formation of

mixed aggregates. Mixture of sintanol DS-10 with lipase have the highest solubilizing ability due to large effective volume of associates. Compositions of surfactants sintanol DS-10–sintamid-5 (70:30) and surfactant's mixture–lipase (with lipase content of 30%) can be recommended as deresination agents.

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