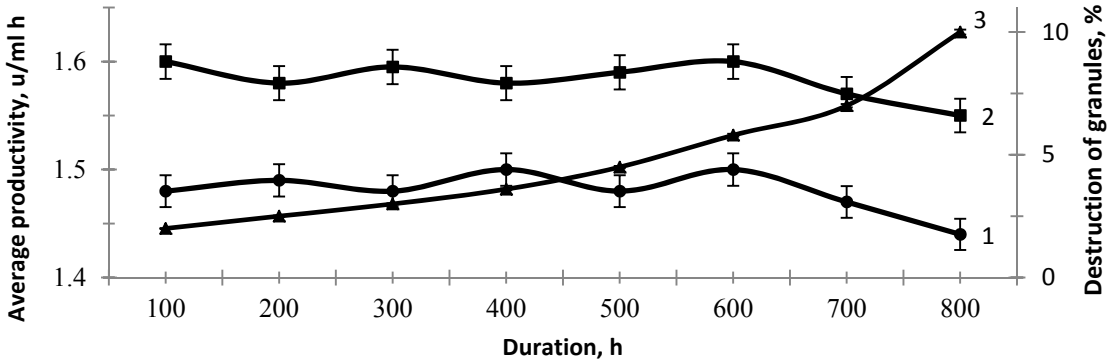

Figure 4 – The influence of duration of using cells of strain *A. awamori F-0719 RKM* in immobilized state (Curves denominations: 1 - cultivating in nutrient medium I; 2 - cultivation in nutrient medium II; temperature - 28°C; without mixing; 3 – destruction of the granules in nutrient medium II)

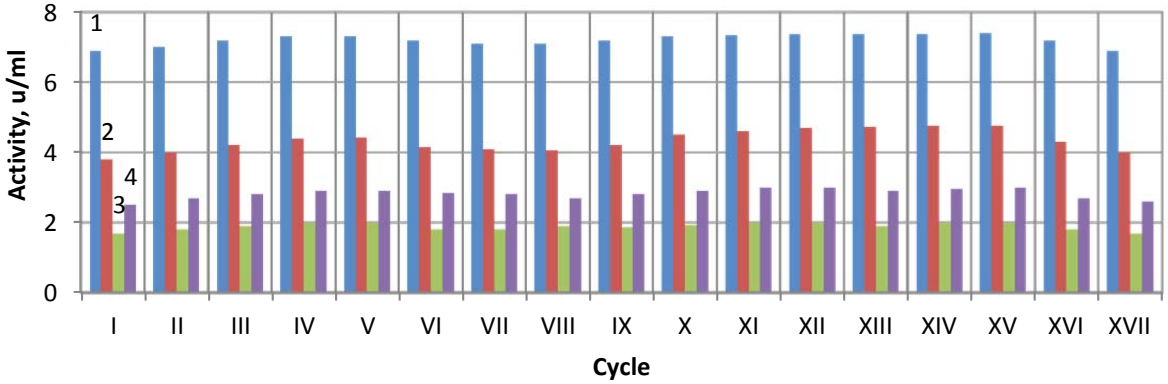

Figure 5 – Activity of the enzyme complex in case of repeated usage of immobilized strain *A. awamori F-0719 RKM* in the process of maceration. The duration of one working cycle of immobilized biocatalyst is 12 hours (the optimum duration of maceration). (Designations curves, enzymes activity, u/ml: 1 - pectolitic; 2 – polygalacturonic; 3 – pectinesterase; 4 - pectin lyase)

It has been experimentally established (Figure 4) that the average pectolitic activity in the cultural fluid during cultivation in nutrient medium I is approximately 1.5 u/ml×h 1.6 u/ml×h when cultured in medium II for 600 hours, then it starts decreasing.

The thus prepared highly active immobilized biocatalyst based on cryogel of polyvinyl alcohol at the concentration of 100 g/l containing cells of strain *A. awamori F-0719 RKM* in the initial concentration of 0.8 g/l is characterized by long and stable biosynthesis of a complex of pectolitic enzymes in the process of maceration (Figure 4, 5). The maximum pectolitic activity of the developed immobilized biocatalyst is 7.4 u/ml. In the production conditions, activity of immobilized biocatalyst started decreasing after the 17-th cycle, i.e. its use with the maximum efficiency is limited to 200-220 hours.

For the purpose of assessing the efficiency of obtaining pectinases, Table 1 shows the comparative characteristics of the conditions and the products of cultivating free and immobilized cells of mycelium.

Table 1 – Comparative characteristics of the conditions and the products of cultivating free and immobilized cells of *A. awamori F-0719 RKM* in nutrient medium I

Parameters of cultivation	Free cells	Immobilized cells
Duration of cultivation, h	84	600 (220)*
The maximum duration of pectinase synthesis, h	70-80	Continuously for 600 (220)*
Pectolitic activity, u/ml	2.10	7.40
Polygalacturonic activity, u/ml	1.25	4.77
Pectinesterase activity, u/ml	0.90	2.00
Pectin lyase activity, u/ml	1.30	2.95
Specific activity, u/mg of protein, u/mg of immobilized biomass	87.3	465

Note: *600 hours – laboratory conditions, 220 – production conditions in a thermal vinificator with stirring at the temperature of 45 °C

The obtained experimental data indicate the advantages of using cells of *A. awamori F-0719 RKM* in immobilized state, a 3.5 times increase in the biosynthesis of a complex of pectolitic enzymes, and a 5.3 times increase in the specific activity allow efficient and repeated use of immobilized cells of the producer as the source of pectolitic enzymes in wine production. In addition, the obtained immobilized biocatalyst is characterized by markedly elevated polygalacturonic and pectin lyase activities, which are aimed at releasing the coloring and phenolic substances from the peel of grapes, and at destruction of pectin and facilitating of grape pomace pressing.

Efficiency of immobilized cells of *A. awamori F-0719 RKM* for high-quality table red wines has been studied. Before the first use, the biocatalyst was subjected to aging in nutrient medium I for 10 hours. The used method of thermal vinification provides a higher flexibility. First, the processes of extraction and fermentation are separated, since colored must is fermented without the pomace. Secondly, the temperature profiles may be adjusted; if necessary, the grapes partially affected by mold may be successfully processed, which is impossible with the use of classic pomace fermentation. Third, multi-variance and route of processes are easily resolved. The use of thermal vinification ensures high economical efficiency, the route of the process with complete mechanization and automation of operations, inactivation of harmful microorganisms, reduces the dosages of sulfitization and high quality of red table wines. The choice of the duration of maceration is explained by the fact that in case of a prolonged contact of pomace with the developed immobilized biocatalyst (over 12 hours) wine materials become oversaturated with polyphenols, which tinctures excessive astringency and roughness to the taste of wine. If maceration continues for less than 10 hours, less significant changes in transparency and viscosity are observed, which indicates insufficient hydrolytic processes. During the research, the main indicators that characterize efficiency of enzyme preparations have been determined (Table 2).

Based on the obtained data for the production of red table wines, immobilized biocatalyst in the concentration of 11 g of granules/l of pomace may be recommended.

Thus, a conclusion may be made about the efficiency of using immobilized cells of *A. awamori F-0719 RKM* in wine production. The use of the obtained immobilized biocatalyst ensures an increased yield of must from grape pomace, facilitates pressing of grape pomace and brightening of wine due to decreasing viscosity and the content of solids in the must, and obtaining wine materials enriched in colorants and phenolic substances.

Figure 6 shows a diagram of a comparative tasting assessment of the studied red table wines after enzymatic treatment with immobilized cells with the reference samples.

The wines obtained as a result of enzymatic treatment are characterized by rich, complex aroma and intense, harmonious, styptic taste, which is the result of deep hydrolysis of polyphenols.

Table 2 - The main indicators that characterize efficiency of using immobilized cells of *A. awamori F-RKM 0719

Indicators	Reference	Dosage of fermentation preparations, granules / l of pomace					
		5	6	7	9	11	12
Overall yield of must and the yield of self-flowing must, ml	560 (410)	665 (417)	671 (422)	678 (431)	682 (430)	687 (437)	687 (438)
Suspended solids, g/100 ml	3.2	2.8	2.7	26	2.4	2.3	2.3
Relative viscosity	1.61	1.56	1.51	1.46	1.43	1.40	1.41
Phenolic compounds, mg/l	1.830	1.925	1.970	2.032	2.095	2.180	2.180
Anthocyanins, mg/l	520	528	579	593	615	675	672
Color intensity	1.19	1.20	1.22	1.24	1.25	1.26	1.25
Tint	1.09	1.06	1.02	0.99	0.96	0.94	0.95

Note: *tenfold reuse of the biocatalyst, time of maceration is 12 hours

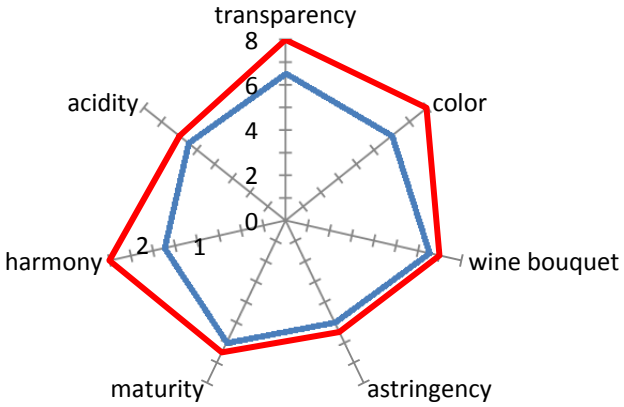

Figure 6 - Organoleptic diagram (Designation: 1 - reference; 2 – immobilized cells of *A. awamori F-RKM 0719)

CONCLUSION

The advantages of the method of immobilization based on the polyvinyl alcohol cryogel are in the fact that the polyvinyl alcohol has no negative impact on pectolitic enzymes biosynthesis by immobilized cells; on the contrary, viability and enzymatic activity of the latter increase due to the porous structure of the matrix of the carrier (pores cross-section is 0.5-1.2 μm), which ensures easy diffusion of components of the nutrient medium and the metabolic products. In addition, the polyvinyl alcohol cryogel is a visco-elastic non-friable material which is not subject to abrasive wear, has good characteristics during long-term cultivation (up to 600 h), and can accept any form of granules, which is suitable for various reactors with various operating profiles. The developed biocatalyst has significantly increased its productivity in terms of pectolitic activity (7.4 u/ml), compared to free cells. The use of immobilized cells of *A. awamori F-RKM 0719* in obtaining red table wines is economically and technologically substantiated, since due to the possibility of using them repeatedly (up to 17 times), the actual consumption is 10 times less than that of the conventional enzyme preparations, with the cost of its cleaning being comparable with the cost of producer immobilization. The enzyme-heat treatment during the process of maceration significantly increases the yield of highly colored must, improves the process of lightening and filtration of must, and ensures the maximum extraction of anthocyanins and phenolic compounds, which allows obtaining harmonious red wines with a strong fruit notes in the high quality aroma.

REFERENCES

[1]. Dontsov, A.G., Shubakov, A.A. *Pectolytic Enzymes: Purification, Activation, Microbiological Synthesis.* Ural Branch of Russian Academy of Science, Yekaterinburg, 2010, pp. 163.

[2]. Ajayi, A.A., Osunlalu, E.O., Peter-Albert, C.F., Adejuwon, A.O. Studies on pectinolytic and proteolytic enzymes from deteriorated grapes (Vitis vinifera). *Covenant Journal of Phsical and Life Sciences*. 2014, 1(2), 1-15.

[3]. Mieszczakowska-Frąc, M., Markowski, J., Zbrzeźniak, M., Płocharski, W. Impact of enzyme on quality of blackcurrant and plum juices. *Food Science and Technology.* 2012, 49, 251-256.

[4]. Sandri, I.G., Fontana, R.C., Barfknechk, D.M., Silveira, M.M. Clarification of fruite juices by fungal pectinases. *Food Science and Technology*. 2011, 44, 2217-2222.

[5]. Markosov V. A., Ageeva N. M., Chaplygin, A. V. *Issledovanie vliyaniya tehnologii proizvodstva krasnih stolovih vin na kontsentratsiyu antotsianov i fenolokislot* [Studying the influence of the technology of red table wines production on the concentration of anthocyanins and phenolic acids]. Editorial body of magazine "Bulletin of food technology HEIs", Krasnodar, 2010, pp. 6

[6]. Ageev, N. M., Markosov, V. A. Vliyaniya fermentnih preparatov na sostav aromatoobrazuyuschih komponentov v krasnih stolovih vinah [Influence of enzyme preparations on the composition of the aroma forming components in red table wines]. *Wine production and viniculture*. 2013, 3, 19 – 22.

[7]. Romero-Cascales, I., Ros-García, J.M., López-Roca, J.M., Gómes-Plaza, E. The effect of a commercial pectolytic enzyme on grape skin cell wall degradation and colour evolution during the maceration process. *Food Chemistry.* 2012, 130, 626-631.

[8]. Mekhuzla N. N., Sherbakov S. S., Semenova M. V., Sinitsyn A. P. Fermentnie preparati otechestvennogo proizvodstva dlya polucheniya prirodno-polusladkih vin [Domestic enzyme preparations for obtaining natural semi-sweet wines]. *Wine production and viniculture*. 2010, 4, 10-11.

[9]. Mojsov, K., Ziberoski, J., Bozinovic, Z. The effect of pectolytic enzyme treatments on red grapes mash of Vranec on grape juice yields. *International Cross-Industry Journal*. 2011, 7(1), 84-86.

[10]. Pedrolli, D.B., Monteiro, E.Gomes, E.C. Carmona, Pectin and pectinases: production, characterization and industrial application of microbial pectinolytic enzymes. *Open Biotechnol. J.* 2009, 3, 9-18.

[11]. Kurbatova E. I., Sokolova E. N., Rimareva L. V. Ispolzovanie preparatov, poluchennih iz glubinnoi kulturi A.foetidus MB-4 dlya gidroliza klyukvi [Using preparations obtained from submerged culture of A. foetidus MB-4 for cranberry hydrolysis]. *Production of alcohol and liquors.* 2005, 3, 13-14.

[12]. Semenova M. V., Grishutin S. G., Gusakov, A.V., Okunev, O. N., Sinitsyn A. P. Videlenie i svoistva pektinaz iz griba Aspergillus japonicus [Extraction and properties of pectinases from fungus Aspergillus japonicus]. *Biochemistry*. 2003, 68(5), 686-697.

[13]. Nedovic, V., Willaert, R. *Fundamentals of cell immobillisation biotechnology.* Kluwer Academic Publ., Dordrecht, 2004, pp. 555.

[14]. Efremenko, E.N., Lyagin, I.V., Senko, O.V., Stepanov, N.A., Spiricheva, O.V., Azizov, R.E. *Modern trends in application of immobilized cells for environmental bioremediation*. In book: Leading-Edge Environmental Biodegradation. Nova Science Publ. Inc., New York, 2007, pp. 11-51.

[15]. Navrátil, M., Dömény, Z. Hronsky, V., Sturdik, E., Smogrovocová, D., Gemeiner, P. Use of bioluminometry for determination of active yeast biomass immobilized in ionotropic hydrogels. *Anal. Biochem.* 2000, 284(2), 394-400.

[16]. Efremenko E. N., Tatarinova N. Y. Vliyanie dlitelnogo hraneniya kletok mikroorganizmov, immobilizovannih v kriogel polivinilovogo spirta, na ih vizhivaemost i biosintez tselevih metabolitov [The influence of long-term storage of microbial cells immobilized in cryogel of polyvinyl alcohol on their survival and biosynthesis of target metabolites]. *Microbiology.* 2007, 76 (3), 383-389.

[17]. Sinitsyn, A. P., Rainina, E. I., Lozinsky, V. I., Spasov, S. D. *Immobilizovannie kletki mikroorganizmov* [Immobilized cells of microorganisms]. Ed. House of MSU, Moscow, 1994, pp. 288.

[18]. Shaskolskiy, B. L., Ivanov, R. V. *Razrabotka polimernih kriogelei dlya polucheniya materialov biotehnologicheskogo naznacheniya* [Development of polymeric cryogels for production of biotechnological materials]. Proceedings of the VII Scientific conference of young scientists, postgraduates and students "Materials and technologies of the XXI century". Kazan, 2007, pp. 137.

[19]. Lozinsky V. I. Kriotropnoe geleobrazovanie rastvorov polivinilovogo spirta [Cryotrophic gelation of polyvinyl alcohol solutions]. *Achievements in chemistry.* 1998, 67(7), 641-655.

[20]. Dzhakasheva, M.A., Kedelbayev, B.S. Getting the active strain of *Aspergillus awamori* – pectinase produser. *International journal of applied and fundamental research*. 2014, 11(4), 593-597.

[21]. Lozinsky, V. I., Zubov, A. L. Ustroistvo dlya formirovaniya granul [An apparatus for forming granules]: Patent No. 2104866, Russia, IPC B29B9/10, B01J2/02. Appl. 02.09.1996. Publ. 20.02.1998.

[22]. Perth S. D. Osnovy kultivirovaniya mikroorganizmov i kletok [Fundamentals of cultivating microorganisms and cells]. Mir, Moscow, 1978, pp. 331.

[23]. Metody tehnohimicheskogo kontrolya v vinodelii [Methods of technochemical monitoring in wine production]. Tavrida, Simferopol, 2002, pp. 260