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INFLUENCE OF PERFLUORODECALIN WITH GAS TRANSPORT FUNCTION ON GROWTH OF MICROORGANISMS

Abstract. In the work influence of perfluorodecalin on growth and development of microorganisms of different groups is investigated. Entering into liquid nutrient mediums perftororganical connections with gas-transport function is shown, that, in particular perfluorodecalin in concentration of 0.2-2 % provides a gain of a biomass of microorganisms. The opportunity of use of perfluorodecalin in biotechnological processes of cultivation bacteria of Rhodococcus, Escherichia, Pseudomonas and Bacillus and micomicetes Penicillium, Fusarium and Saccharomyces is shown. Investigation of influence of perfluorodecalin in Chapek-Dox media upon change of micromycethes biomass has a certain interest. Results of experiments testify that the maximal content of biomass quantity have been noted in each 48-96 hours at the growth of culture of micromycethes P. chrysogenum MB 104, F.moniliforme BУ 245, F.graminearum 534, S. cerevisiae K on Chapek-Dox media with addition of 2 % of perfluorodecalin, concentration peak of biomass on the media without perfluorodecalin have been obtained on 48 hours later. Herewith maximum values of the biomass on media with perfluorodecalin in 2 times (penicill and fuzaries) exceed the maximums, reached on ambience without perfluorodecalin.

Key words: perfluorodecalin, microorganisms, biotechnological processes, gas-transport function, Chapek-Dox media.

Introduction. Lately development and putting into practice of modern technologies of microorganisms cultivation, obtaining of the microbe biomass and biological active compounds - products of activity of bacteria and micromycethes, have the major practical meaning. Nowadays the various nourishing products, protein preparations, amino acids, organic acids, spirits, physiological active substances - antibiotics, enzymes, hormones, facilitators of the growing, vaccines against infectious diseases of the person and animal, facilities of the fight with insect and rodent - a vermin of the agriculture are obtained by help of microorganisms.

The majority of microorganisms using by human in biotechnology for growth, development and production of biological active substances need oxygen for providing of high energetical exchange [1]. Oxygen is the most important elements using by the microorganisms for building of structural components of microbial cell, for energy obtaining, and it participates in various biochemical reactions, providing the processes for the microbe activity. Provision by oxygen or it's removal for anaerobes, as well as removal of exhaust fumes and metabolism products is one of the most important factors of efficiency of biotechnological processes, defining the productivity of their biomass accumulation and synthesis of biological active substances.

The majority of microorganisms using by human in biotechnology need oxygen during processes of development. It is known that more high output of target product is provided by the deeply cultivation in fluid nourishing media. For normal growth of aerobes in deep layer of media the aeration and improvement of condition for transport gaz into microorganisms cells are required [2].

Putting into medical practice of the new class of efficient and safe preparations with gaz transport function on the base of perftororganic compounds (PFOC) is typical for the end of 21th century. As a result of multiple investigations the variety of new preparation including Oxygent, Therox, Oxyfluor (USA); Fluosol-DA (Japan); Emulsion II (China); Perftoran (Russia) has been created. Perftororganic compounds have complex of practically useful properties: high chemical and biological stability, absence of toxity for living organisms, abilities to dissolve gases (until 50 % O_2, 200 % CO_2, 300 % C_2H_6 etc.) and change the permeability of the cell's membranes, herewith relieving transport of substances [3].

The review of the literature has shown that at present PFOC have usage in technique, medicine, cosmetologies, but usage of that class of compounds in biotechnology doesn't have discussion and realization. In this connection study of possibility of usage of fluid PFOC in composition of nourishing media for improvement of gastransport process of microorganisms cultures has the big interest.

The purpose of the present work is an estimation of possibility of usage of perfluorodecalin with gas transport function in biotechnology for improvement of cultivation processes of practically useful microorganisms.

Methods

The influence of putting into cultural media the various concentration of Perfluorodecalin on the growth of bacteria and microscopic fungi (micomycethes) have been investigated. Rhodococcus erythropolis BY 43, Escherichia coli M 17, Pseudomonas putida PP 44, Bacillus subtilis 3 from bacterial cultures, from micromycethes the strains of Penicillium chrysogenum MB 104, Fusarium moniliforme BY 245, Fusarium graminearum 534, Saccharomyces cerevisiae K have been used. Culture of given microorganisms have been choosen, because in biotechnology at production of practical useful products the big amount of strains of this species are used for gained of expensive antibiotics, eybiotics, micoproteins, food products, facilitators of the growing of plants - hibberelines. Pseudomonas and rodococcus are used as the kxenobiotics of biodestructirs.

For growth of bacteria and micromythetes the meat-peptone bouillon and standard media: agar Chapek, media Chapek-Dox have been used [4]. For growing of streptomythetes and fuzariouz fungi the fluid nourishing media that contain starch - 3,0 %: $(NH_4)_2C_4H_4O_6$ - 0,05 %, $(NH_4)_2SO_4$ - 0,4 %; $CaCO_3$ - 0,8 %; K_2HPO_4 - 0,01 %; glucose - 1,5% (entered in sterile nourishing media); water until 100 % have been used. Cultivation are carried out in Erlenmeyer flask with capacity of 250 sm^3 and volume of fluid nourishing media 80 sm^3, that contains sowing cultures, corresponding strain, and from 0,2 to 2,0 % Perfluorodecalin. Growing have been carried out at the 27 °C and constant mixing with velocity of the rotation 230 rotations per minute, culture in flasks with media without Perfluorodecalin served as a checking culture in all experiments. The tests sampled in each 8 hours on 2 ml for determination of quantity alive cells of Pseudomonas and Rodococcus in cultural media by method of sowing of serial breedings on thick nourishing media on the base of meat-peptone agar (MPA) (table 1).

Table 1 - Growth of culture of bacteria Pseudomonas putida, Rhodococcus erythropolis, Escherichia coli and Bacillus subtilis on the meat-peptone bouillon (MPB) with addition of perfluorodecalin

Strain	Content of perfluorodecalin in media, %	Quantity of alive bacteria/sm^3 in each: hours from beginning of cultivation ($\cdot 10^6$)				
		0	12	24	36	48
P. putida PP 44	1. 0	45	96	1899	5029	4340
	2. 0,2	45	163	6986	5732	4433
	3. 2,0	45	251	8475	6348	4900
R. erythropolis BY 43	4. 0	42	77	1087	2438	2295
	5. 0,2	42	85	1584	2953	2564
	6. 2,0	42	97	3948	3732	2325
E. coli M 17	7. 0	43	109	2436	6423	5563
	8. 0,2	43	112	2662	7484	5431
	9. 2,0	43	125	8425	6617	5042
B. subtilis 3	10. 0	52	74	985	2261	2138
	11. 0,2	52	77	1112	2532	2315
	12. 2,0	52	80	2933	2397	2254

Results and discussions

Results of experiment, presented in the table 1, testify that addition of 0,2 % Perfluorodecalin into media brought about reinforcement of the growth of bacteria under investigation in comparison with control that particularly is distinctly noted under growth of P. putida. At growing of the bacteria cultures P. putida, R. erythropolis, E. coli and B. subtilis on MPA with adding of 2 % perfluorodecalin already maximum contents of quantity of alive bacteria were noted in each 24 hours in media.

Investigation of influence of Perfluorodecalin in Chapek-Dox media upon change of micromycethes biomass has a certain interest. Results of experiments presented in the table 2 testify that the maximal content of biomass quantity have been noted in each 48-96 hours at the growth of culture of micromycethes P. chrysogenum MB 104, F. moniliforme BУ 245, F. graminearum 534, S. cerevisiae K on Chapek-Dox media with addition of 2 % of Perfluorodecalin, concentration peak of biomass on the media without Perfluorodecalin have been obtained on 48 hours later. Herewith maximum values of the biomass on media with Perfluorodecalin in 2 times (penicill and fuzaries) exceed the maximums, reached on ambience without Perfluorodecalin.

Results presented in tables 1 and 2 are an average arithmetical results on the 3 definition of content of biomass and concentration of alive bacteria. Degree of the deflection of the determination from average arithmetical did not exceed 15 %.

Results of experiments testify that entering of Perfluorodecalin in culture brought to intensification of change of mass characteristics, that is confirmed by increase of velocity of growth and increase of biomass output or absolute quantity of microorganisms.

The expenses on use of PFOS under cultivation of bacteria and micromycethes will be profitable because of the more high output of the target products.

Table 2 - Change of biomass of micromycethes at cultivation on Chapek-Dox media with addition of Perfluorodecalin

Strain	Content of Perfluorodecalin in media, %	Biomass of micromycethes (mg %) in Chapek-Dox media in the process of growth					
		0	48	96	144	192	240
P. chrysogenum MB 104	0	36	259	705	816	794	745
	2,0	36	682	1656	1518	1356	880
F. moniliforme BУ 245	0	48	272	764	858	803	731
	2,0	48	743	2145	1652	1493	976
F. graminearum 534	0	42	264	687	795	783	645
	2,0	42	729	1964	1421	1245	927
S. cerevisiae K	0	65	440	497	220	204	134
	2,0	65	632	518	321	242	128

Conclusion. Perfluorodecalin in concentrations from 0,2 % to 2 % promoted the intensification of growth in comparison with control. So, results of the investigation of the influence of Perfluorodecalin on growth and development of microorganisms of the various taxonomic groups has shown that entering of PFOS with gas transport function, in particular Perfluorodecalin in concentrations of 0,2-2 % into fluid nourishing media, provides the increase of the biomass of microorganisms. Possibility of usage of Perfluorodecalin in biotechnological processes of cultivation of bacteria of Rhodococcus, Escherichia, Pseudomonas, Bacillus and micromycethes of Penicillium, Fusarium and Saccharomyces has been shown.

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МИКРООРГАНИЗМДЕРДІҢ ӨСУІНЕ ГАЗ ТАСЫМАЛДАУ ҚЫЗМЕТІ БАР ПЕРФТОРДЕКАЛИННІҢ ӘСЕРІ

Аннотация. Жұмыста әртүрлі микроорганизмдердің өсуіне және дамуына перфтордекалиннің әсері зерттелген. Перфтордекалиннің микроорганизмдерді тереңдетіп өсіргендегі газ тасымалдау функциясын атқаруы және биосинтез процесінің жылдамдауына әсері көрсетілген. Rhodococcus, Escherichia, Pseudomonas мен Bacillus бактериялық туыстығын және Penicillium, Fusarium and Saccharomyces микомицеттерден тереңдетіп өсірудегі газ тасымалдау функциясына перфтордекалинді 0,2-2% пайдалану мүмкіндігінің бар екендігі тәжірибелік түрде дәлелденді. Микромицет биомассасының өзгеруіне Чапек-Докс ортасында перфтордекалиннің әсері зерттелген. P. chrysogenum MB 104, F. moniliforme ВУ 245, F. graminearum 534, S. cerevisiae К микромицет культуралардың 2% перфтордекалині бар Чапек-Докс ортасында өсу кезінде 48-96 сағаттан кейін биомассаның максимальды мөлшері байқалды, ал перфтордекалині жоқ ортада биомасса концентрацияның максимумы барлық жағдайларда уақыт бойынша 48 сағатқа кешіктіріп түзілген. Бұл кезде перфтордекалині бар ортасында биомассаның максималды мәндері (пеницилл және фузарийлер үшін) перфтордекалині жоқ ортадағы максимумдарымен салыстырғанда 2,0 есе жоғары болды.

Тірек сөздер: перфтордекалин, микроорганизмдер, биотехнологиялық процестер, газ тасымалдау қызметі, Чапек-Докс ортасы.

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ВЛИЯНИЕ ПЕРФТОРДЕКАЛИНА С ГАЗОТРАНСПОРТНОЙ ФУНКЦИЕЙ НА РОСТ МИКРООРГАНИЗМОВ

Аннотация. В работе изучено влияние перфтордекалина на рост и развитие микроорганизмов различных групп. Результаты исследования влияния перфтордекалина на рост и развитие микроорганизмов разных таксономических групп показали, что внесение в жидкие питательные среды перфторорганических соединений с газотранспортной функцией, в частности перфтордекалина в концентрации 0,2-2% обеспечивает прирост биомассы микроорганизмов. Показана возможность использования перфтордекалина в биотехнологических процессах культивирования бактерий родов Rhodococcus, Escherichia, Pseudomonas и Bacillus и микромицетов родов Penicillium, Fusarium и Saccharomyces. Определенный интерес представляло исследование влияния перфтордекалина в среде Чапека-Докса на изменение биомассы микромицетов. Результаты экспериментов свидетельствуют, что при росте культур микромицетов P. chrysogenum MB 104, F.moniliforme ВУ 245, F.graminearum 534, S. cerevisiae К на среде Чапека - Докса с добавлением 2 % перфтордекалина уже через 48 - 96 часов культивирования было отмечено максимальное содержание количества биомассы, при этом пик концентрации биомассы на среде без перфтордекалина был получен во всех случаях на 48 часов позже. При этом максимальные значения биомассы на среде с перфтордекалином в 2,0 раза (для пеницилл и фузариев) превышали максимумы, достигаемые на среде без перфтордекалина.

Ключевые слова: перфтордекалин, микроорганизмы, биотехнологические процессы, газотранспортная функция, среда Чапека-Докса.