SELF CONTAINED BIOREACTOR USAGE FOR SMALL-SCALE MICROBIAL PESTICIDES PRODUCTION

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Abstract: Present work reports bioreactor AF-0.170 test results. This novel bioreactor was designed in ETI “Biotechnica” of NAAS for small-scale microbial pesticides production on the base of biolaboratories and biofactories. Cell concentration in culture liquids of microbial preparations based on *Beauveria Bassiana* and *Pseudomonas fluorescens* obtained in tests had an order of 10^9 CFU·ml⁻¹ and contamination level was not exceed 0.3%. Results analyses demonstrated 2.4 times increasing of preparation yield per cycle combined with moderate decreasing of cell density in culture liquid for submerged fermentation in bioreactor versus shaker technology. Advantages of self contained bioreactor usage for small-scale scale microbial pesticides production at Ukrainian and Moldovan biofactories are shown.

Key words: bioreactor, culture liquid, microbial pesticides, submerged fermentation, titer

Introduction

Small-scale microbial pesticides production at Ukrainian and Moldovan biofactories and laboratories is still based on industrial microbiological shaker technology [1]. This technology allows obtaining guaranty high quality culture liquids of microbial preparations, but in the other hand such processing technique is too labor-intensive [1, 2]. One of the ways of solving this problem lies on technology changing from shaker usage to submerged fermentation in bioreactors.

However specialized bioreactors for manufacturing of microbial pesticides are not produced by Ukrainian as well as Moldovan industry. They can be potentially substituted by industrial fermenters and chemical reactors. But such reactors with 50-200L total volume required for small-scale production of microbial pesticides are too energy-consuming in operation [1]. In light of this, the creation of bioreactors oriented on small-scale microbial pesticides production is important today.

Such bioreactors and fermentation technology have been worked out in Engineering and Technological Institute (ETI) “Biotechnica” of NAAS of Ukraine since the 2000s [2]. The recent researches in this field were focused on developing of self contained bioreactor suitable for bacteria and fungi seed cultures cultivation for biofactories based on Fermentation Complex technology [2, 3] and small-scale production of microbial pesticides in laboratories [4]. The bioreactor is meant for usage in several manufacturing technological stages: liquid nutrient medium making, sterilization and further submerged fermentation.

This work reports recent results of self contained bioreactor AF-0.170 using for different microbial pesticides production.

Materials and Methods

Self contained bioreactor AF-0.170 was used for submerged fermentation of batch cultures. The bioreactor consisted of fermentation tank (170L) with mixer and air sprayer; water jacket; hatch with mixer driver, access hole and inoculum valve; control cabinet (Fig. 1).

Fig. 1. The schematics (a) and photo (b) of self contained bioreactor AF-0.170.

1 – case, 2 – fermentation tank, 3 – water jacket, 4 – turbine mixer, 5 – air sprayer, 6 – hatch, 7 – inoculum valve, 8 – platform

Slow-speed electrically driven turbine mixer (75 rpm) was used for agitation. The aeration was realized by bubbling of pressured air through the sprayer into fermentation medium. Tubular heating elements (12 kW) located in water jacket were used for liquid medium's heating while sterilization. Cooling of sterilized nutrient medium was based on circulation of water from bioreactor jacket through external cooling circuit with water tank (2 m³).

There were two microbial pesticides produced in bioreactor in tests: Boverin BT and Planriz BT. Boverin BT is a fungi preparation on the base of *Beauveria Bassiana* st. 71661, and Planriz BT is a bacteria preparation based on *Pseudomonas fluorescens* st. AP-33 [2]. Both cell cultures were cultivated on liquid nutrient mediums on the base of corn essence and sugar beet molasses. Nutrient medium making process took place directly in fermentation tank. Final acidity of the medium was $pH = 6.5$ for fungi and $pH = 7.3$ for bacteria. The batch of nutrient medium (120L) was sterilized at 120°C during 60 minutes. Respective liquid seed culture at a rate of 12L was added into fermentation tank after medium cooling process. Fermentation lasted for 72h for fungi and 48h for bacteria. The temperature regimes of cultivation were 27°C for *Beauveria Bassiana* and 29°C for *Pseudomonas fluorescens*. Cultured liquids obtained in the tests were analyzed within the samples microscopy [5]. Also the verification by serial dilutions on selective media was made [2].

Results and Discussion

The bioreactor test results are listed in the table 1. As one can see obtained biopreparations based on fungi *Beauveria* as well as bacteria *Pseudomonas* have adequate quality for microbial pesticides. Microbial purity for both pesticides is 99.7% and final cell density has an order of 10^9 CFU·mL⁻¹. It should be pointed that such titer value differs only at one order from the same cultures obtained by means of industrial shaker technology (table 2) [2]. That is not so much as it can be for scaling-up from 3L shaker flask to 170L fermentation tank [5].

Table 1

The test results of microbial pesticides manufacturing in self contained bioreactor AF-0.170

No	Biopesticide name	Basic cell culture	Culture liquid yield per cycle, L	Fermentation time, h	Titer, CFU·mL ⁻¹	Culture liquid contamination level, %
1	Boverin BT	<i>Beauveria Bassiana</i>	132	72	$5.0 \cdot 10^9$	0.3
2	Planriz BT	<i>Pseudomonas fluorescens</i>	132	48	$8.0 \cdot 10^9$	0.3

Table 2

The comparison of fermentation technologies applied for small-scale microbial pesticides production

No	Biopesticide characteristics	Fermentation technology for microbial pesticides production	
		Submerged fermentation in bioreactor AF-0.170	Cultivation on industrial shaker KPM 36/90 T
1	Boverin BT		
1.1	Fermentation time, h	72	72
1.2	Titer, CFU·mL ⁻¹	$5.0 \cdot 10^9$	$3.0 \cdot 10^{10}$
1.3	Culture liquid yield per cycle, L	132	54
2	Planriz BT		
2.1	Fermentation time, h	48	48
2.2	Titer, CFU·mL ⁻¹	$8.0 \cdot 10^9$	$4.0 \cdot 10^{10}$
2.3	Culture liquid yield per cycle, L	132	54

Comparative analysis of fermentation technologies features (table 2) shows that bioreactor using leads to significant increasing of preparation yield per cycle (in 2.4 times) against to low decreasing of cell density in culture liquid. This in combination with significant manual labor decreasing proves that submerged fermentation in bioreactor is the most attractive technology for small-scale microbial pesticides production.

Obtained results also illustrate practicability of engineering solutions made while bioreactor designing. Significant cell density in culture liquids achieved in tests shows that medium agitation can be realized by slow rotating turbine (75 rpm). As a comparison, rotation velocity of standard mixers for industrial bioreactors and fermenters lies in 100-1000 rpm [5]. Intensification of agitation process in our case is achieved by special aeration technique in which air supplied to low pressure area inside rotating turbine and then extended over the whole fermentation medium volume due to centrifugal force.

Also it is clear that some technological processes need to be enhanced. Sterilized medium cooling process for example must be time cut. It will lead to significant energy save and processing time decrease. It should be noted that sterilized nutrient mediums cooling process, as usual, is one of the most expensive and problem area in fermentation technology [1, 2, 4]. Solving this problem for self contained bioreactor will be one of the aims of our further work.

Conclusions

Self contained bioreactor AF-0.170, designed in ETI “Biotechnika” of NAAS, is a good alternative to industrial shaker technology for small-scale microbial pesticides production at Ukrainian and Moldovan biofactories. Its usage allows high quality preparations obtaining with cell densities of 10^9 CFU·mL⁻¹ and permits culture liquid yield per cycle increasing more then two times in comparison with standard industrial shaker technology.

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Аннотация

В статье приведены результаты испытаний нового биореактора, разработанного в ИТИ «Биотехника» НААН для малотоннажного производства микробиологических средств защиты растений на базе биолaborаторий и биофабрик. Показано, что концентрация клеток в культуральных жидкостях препаратов на основе *Beauveria Bassiana* и *Pseudomonas fluorescens*, полученная в испытаниях, составляла порядка 10^9 КОЕ·мл⁻¹, а уровень контаминации не превышал 0,3%. Анализ результатов испытаний показал увеличение выхода препарата за цикл в 2,4 раза при незначительном снижении концентрации клеток в культуральной жидкости для глубинной ферментации в биореакторе по сравнению с качалочной технологией. Показаны и другие преимущества использования автономного биореактора для малотоннажного производства микробиологических пестицидов на украинских и молдавских биофабриках.