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Selection of an Effective Stabilizer for the New Liquid-Phase Biological Product

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Abstract

This paper considers the selection of stabilizing/preserving agents for a long-time storage of the liquid-phase biological product (LBP) used in crop production. LBP belongs to microbiological products, so the authors have been challenged to preserve its activity by reaching three objectives: to keep the number of useful microflora close to the original one, prevent the growth of sanitary indicator microorganisms (enterobacteria) and avoid a bad smell caused by active microflora.

The work has been performed in three experiment series. Experiment Series 1 and 2, which are different by time (six months and a year), have been devoted to the studying of stabilizing properties of some preservatives such as sodium benzoate, sodium thiosulfate, aminobenzoic acid and sodium chloride. Experiment Series 3 has tested LBP (of different storage durations) in spring wheat germination. It has been found that the best LBP quality is guaranteed by 5% (mass to mass) sodium chloride at low storage temperatures. The storage duration for LBP containing the stabilizer should not exceed six months.

Keywords: liquid-phase biological product for crop production, stabilization method

1. Introduction

Stabilizers or preservatives are an integral part of most up-to-date products. Basically these substances (aimed to keep products fresh and intact) are used in food, cosmetic and pharmaceutical industries. There is a wide range of preservatives of different nature, activity type or bacteriostatic effect used. Mind that no universal stabilizer or preservative exists, so manufacturers sometimes use a mix of different preservatives, and they can differ strongly in each particular case.

Stabilizers or preservatives significantly affect finished product's microflora. They either make it grow or multiply slower, or exterminate it completely. In this connection, the growing number of different microflora-based products marketed should also be considered as products requiring stabilization of their original properties.

Our All-Russian Research Institute of Reclaimed Lands has developed a method for manufacturing of liquid-phase biological products for crop production and agriculture (Rabinovich et al., 2005) that has been improved for some years (Rabinovich et al., 2009). The biological products manufactured by this method were assigned a general name: liquid-phase biological products (LBP). When designing an LBP production line, the authors made it possible to modify the manufactured product quality (Rabinovich et al., 2006) according to product effect tests (Kovalev et al., 2010).

LBP comprises organic and mineral components. Its agent is abundant microflora (up to $n \times 10^{12}$/ml), which can lead to changes in LBP composition and properties, sometimes unfavorable, due to a long-term storage.

The objective of this work is to select the most effective stabilizer for LBP by evaluation of the physicochemical conditions stabilizing long-time LBP composition and properties to be the same or better than freshly made LBP's ones.

2. Method

Achieving the above objectives implied a complex and extensive research. Three series of laboratory experiments were conducted.

Series 1: LBP composition depending on four stabilizers in different concentrations: sodium benzoate (0.001%; 0.005%; 0.010%; 0.015%; 0.020%), sodium thiosulfate (1%; 3%; 5%; 10%), aminobenzoic acid (0.005%; 0.01%; 0.03%; 0.05%) and sodium chloride (5%; 10%; 15%; 20%). The experiment was carried out at two different temperatures: 3 ^{o}C (storage in a refrigerator) and 22 ^{o}C (storage at ambient temperature). Some parameters of LBPs with and without stabilizers (test and reference) were analyzed: at the beginning of the experiment, one month later, three months later and six months later. LBP quality was estimated organoleptically and microbiologically - by the number of N-transforming, phosphate-mobilizing microorganisms, enterobacteria and microscopic fungi (Zvyagintsev, 1991; Szegi, 1983). The experiments were planned so that each test vial was opened only once – for its analysis.

Series 2: LBP composition depending on the most effective stabilizer (selected in Experiment Series 1) used in its optimum concentrations for a longer time – 12 months. The assessment criteria were the same.

Series 3: LBP properties evaluation (for the LBPs selected in Series 2) three months later, six months later, nine months later and 12 months later by physiological testing (*Irgina* spring-wheat seed germination for seven days at 22 ^{o}C). References 1 and 2 were water and freshly made LBP respectively. The experiments were triplicated.

3. Results and Discussion

In case of using preservatives to keep the original composition and properties of liquid-phase biological products for crop production, particularly our LBP, there are no strict requirements comparable with those to, e.g., food stabilizers. Nevertheless, we established our own evaluation criteria and results analyses for preservative effects on the products. So, our analyses of the results obtained emphasized that the preservatives added should not decrease the number of agronomically useful microflora and increase the number of microscopic fungi or enterobacteria (to demonstrate that the LBPs are environmentally safe). It should be noted that – despite the fact that initial LBP contained rather many enterobacteria ($n \times 10^5$/ml) - our stabilized LBP was totally pathogen-free (according to LBP tests by Tver Region Sanitary and Hygiene Laboratory, Russian State Sanitary and Epidemiology Inspectorate).

The next requirement we obeyed when using preservatives in LBP is basic for all preservatives: they must not deteriorate the organoleptical properties of the product preserved (SanPIN, 2003).

Throughout the entire period of storage, LBPs both with and without a preservative kept their original color. After a month-long storage, all LBPs produced sediment. It should be noted that both all references and the majority of stabilized samples developed a bad smell of rotten eggs. The only exclusion was LBP stabilized with sodium chloride. This phenomenon lasted till the end of the experiment.

Since Experiment Series 1 included a great volume of results obtained, this work shows only optimum concentrations of each preservative according to the above requirements (see Table 1). Note that one-month storage of LBP in all variants increased the number of the agronomically useful microflora (the only exception was sodium benzoate at 22 ^{o}C), the latter reaching the maximum grows after three-month storage. It should be noted in this connection that one-month storage of LBP with 5% or 10% sodium chloride as a preservative produced the greatest number of microorganisms – supposedly halophilic ones (Gorshkov & Lipatova, 1987). Greater NaCl concentrations noticeably suppressed the microflora growth throughout the whole experiment compared to other preservatives (not shown in Tables 1 to 3).

Adding each of the preservatives being tested to LBPs and storing the latter at 22 ^{o}C lowered the number of enterobacteria after six months, with the strongest suppression by sodium benzoate. The lowered temperature of LBP storage turned out to be insufficient to meet the enterobacteria number requirements. Using sodium chloride, especially 10%, proved to be an exception. As known (Rabinovich et al., 2009), this NaCl concentration inhibits growth of most microorganisms – including coliforms. There was no microscopic fungi growth observed with each preservative – i.e., each of them met the requirements claimed.

Tables 1 to 3 give convincing evidence that LBP cannot be stored for a long time without preservatives. Nevertheless, considering all our results obtained, it is difficult to unambiguously select a single stabilizer. However, after Experiment Series 1 one can select options of LBP treatment with 10% sodium chloride and further LBP storage in the refrigerator or at ambient temperature, as well as adding 5% sodium chloride and cold storage. It is these options that meet all requirements we claimed. The action of sodium chloride is based on forming an elevated osmotic pressure around bacterial cells, leading to their dehydration, size- and shape-changes, and water metabolism anomalies [8]. Higher NaCl concentrations (15% or 20%) in LBP did not cause any bactericidal effect, but resulted in a strong suppression of all microorganisms tested in the LBP after its one-month storage. Thus, 10% is the top concentration of sodium chloride as a LBP stabilizer.

Experiment Series 2 used 5%; 7.5% and 10% NaCl with LBP storage at 3 °C (Tables 4 to 6). Analyzing the data given in Tables 4 to 6, it is easy to find a clear trend to lowering the number of all tested microorganism groups at the end of the experiments. It turned out impossible to find any definite regularity in the development of different microflora groups depending on the preservative applied.

Table 1. Effect of different stabilizers on the total number of agronomically useful microflora in LBP stored up to six months

Indicator	Storage duration	Reference (LBP without stabilizer)		Stabilizer									
				Sodium benzoate		Sodium thiosulfate		Aminobenzoic acid		Sodium chloride			
		22 °C	3 °C	22 °C (0.001%)	3 °C (0.001%)	22 °C (10%)	3 °C (10%)	22 °C (0.005%)	3 °C (0.05%)	22 °C (5%)	3 °C (5%)	22 °C (10%)	3 °C (10%)
Total number of agronomically useful microflora, CFU/ml ×	Original LBP	15.3×10^{11}											
	1 month	28.2×10^{11}	44.8×10^{11}	10.7×10^{11}	35.7×10^{11}	20.6×10^{11}	17.6×10^{11}	18.8×10^{11}	13.9×10^{11}	57.1×10^{11}	66.2×10^{11}	68.9×10^{11}	43.9×10^{11}
	3 months	29.5×10^{11}	54.8×10^{11}	19.1×10^{11}	86.9×10^{11}	67.0×10^{11}	46.3×10^{11}	26.3×10^{11}	60.7×10^{11}	70.3×10^{11}	49.8×10^{11}	75.3×10^{11}	52.5×10^{11}
	6 months	18.6×10^{11}	48.0×10^{11}	13.2×10^{11}	39.9×10^{11}	30.1×10^{11}	41.6×10^{11}	26.5×10^{11}	47.9×10^{11}	44.8×10^{11}	43.3×10^{11}	70.5×10^{11}	44.9×10^{11}
Requirements claimed are met		+	+	±	+	+	+	+	+	+	+	+	+

Note: CFU/ml × was equal to or greater than the original value throughout the entire LBP storage duration.

Table 2. Effect of different stabilizers on the number of enterobacteria in LBP stored up to six months

Indicator	Storage duration	Reference (LBP without stabilizer)		Stabilizer									
				Sodium benzoate		Sodium thiosulfate		Aminobenzoic acid		Sodium chloride			
		22 °C	3 °C	22 °C (0.001%)	3 °C (0.001%)	22 °C (10%)	3 °C (10%)	22 °C (0.005%)	3 °C (0.05%)	22 °C (5%)	3 °C (5%)	22 °C (10%)	3 °C (10%)
Enterobacteria, CFU/ml**	Original LBP	4.8×10^{5}											
	1 month	8.6×10^{5}	10.0×10^{5}	12.5×10^{4}	5.3×10^{5}	1.1×10^{5}	1.9×10^{5}	0.6×10^{5}	1.8×10^{5}	4.0×10^{5}	1.4×10^{5}	1.7×10^{5}	8.4×10^{4}
	3 months	11.7×10^{5}	19.9×10^{5}	4.0×10^{4}	10.2×10^{5}	3.4×10^{5}	12.4×10^{5}	1.2×10^{5}	13.9×10^{5}	5.4×10^{5}	3.6×10^{5}	3.7×10^{5}	0.5×10^{4}
	6 months	9.0×10^{5}	12.9×10^{5}	0.2×10^{4}	8.5×10^{5}	0.3×10^{5}	2.0×10^{5}	1.2×10^{5}	6.3×10^{5}	3.7×10^{5}	0.8×10^{5}	1.8×10^{5}	0.2×10^{4}
Requirements claimed are met		–	–	+	–	+	±	+	±	±	+	+	+

Note: CFU/ml** was equal to or less than the original value throughout the entire LBP storage duration.

Table 3. Effect of different stabilizers on the smell of LBP stored up to six months

Indicator	Storage duration	Reference (LBP without stabilizer)		Stabilizer									
				Sodium benzoate		Sodium thiosulfate		Aminobenzoic acid		Sodium chloride			
		22 ºC	3 ºC	22 ºC (0.001%)	3 ºC (0.001%)	22 ºC (10%)	3 ºC (10%)	22 ºC (0.005%)	3 ºC (0.05%)	22 ºC (5%)	3 ºC (5%)	22 ºC (10%)	3 ºC (10%)
Smell***	Original LBP	no bad smell											
	1 month	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	no bad smell	no bad smell	no bad smell	no bad smell
	3 months	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	no bad smell	no bad smell	no bad smell	no bad smell
	6 months	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	bad smell	no bad smell	no bad smell	no bad smell	no bad smell
Requirements claimed are met		–	–	–	–	–	–	–	–	+	+	+	+

Note: Smell*** – as the original level throughout the entire LBP storage duration.

Description: All sodium chloride concentrations used promoted the increase in the number of agronomically useful microflora compared to the fresh LBP (original sample), but the number was lower than the reference one (without preservative).

The enterobacteria growth showed a clear dependence: the higher the sodium chloride concentration and corresponding LBP storage duration was, the noticeably lower the number of enterobacteria became.

Table 4. Effect of sodium chloride on the total number of agronomically useful microflora in LBP stored up to 12 months

Indicator	Storage duration	Reference (LBP without preservative)	RM^	Preserved by sodium chloride					
				5%	RM^	7.5%	RM^	10%	RM^
Total number of agronomically useful microflora, CFU/ml ×	Original LBP	10.1×10^{11}							
	3 months	25.2×10^{11}	+	24.1×10^{11}	+	23.5×10^{11}	+	20.9×10^{11}	+
	6 months	23.0×10^{11}	+	19.4×10^{11}	+	17.0×10^{11}	+	18.8×10^{11}	+
	9 months	18.6×10^{11}	+	13.7×10^{11}	+	15.3×10^{11}	+	11.2×10^{11}	+
	12 months	14.2×10^{11}	+	11.1×10^{11}	+	10.7×10^{11}	+	9.0×10^{11}	–

Notes: CFU/ml* – equal to or greater than the original value throughout the entire LBP storage duration; RM^ – requirements to the total number of agronomically useful microflora in LBP are met.

Table 5. Effect of sodium chloride on the number of enterobacteria in LBP stored up to 12 months

Indicator	Storage duration	Reference (LBP without preservative)	RM^	Preserved by sodium chloride					
				5%	RM^	7.5%	RM^	10%	RM^
Enterobacteria, CFU/ml**	Original LBP	2.9×10^5							
	3 months	14.1×10^5	–	1.6×10^5	+	0.8×10^5	+	0.5×10^4	+
	6 months	10.2×10^5	–	0.9×10^5	+	3.1×10^4	+	0.2×10^4	+
	9 months	10.5×10^5	–	2.0×10^4	+	1.1×10^4	+	0.9×10^3	+
	12 months	7.4×10^5	–	0.8×10^4	+	0.3×10^4	+	0.9×10^3	+

Notes: CFU/ml** – equal to or less than the original value throughout the entire LBP storage duration; RM^ – requirements on the number of enterobacteria in LBP are met.

Table 6. Effect of sodium chloride on the smell of LBP stored up to 12 months

Indicator	Storage duration	Reference (LBP without preservative)	RM^	Preserved by sodium chloride					
				5%	RM^	7.5%	RM^	10%	RM^
Smell***	Original LBP	no bad smell							
	3 months	bad smell	–	no bad smell	+	no bad smell	+	no bad smell	+
	6 months	bad smell	–	no bad smell	+	no bad smell	+	no bad smell	+
	9 months	bad smell	–	no bad smell	+	no bad smell	+	no bad smell	+
	12 months	bad smell	–	no bad smell	+	no bad smell	+	no bad smell	+

Notes: Smell*** – as in the original LBP throughout its entire storage duration; RM^ – smell indicator requirements to LBP are met.

Thus, the experimental results obtained demonstrate that application of sodium chloride in all its options (but one) gave the effects required. Consequently, according to the limitations on application of chemical preservatives stated by the Russian sanitary laws (SanPIN, 2003), it is necessary to select such a sodium chloride concentration which is the minimum to achieve the technological effect desired – that is 5%. In this case, LBP preserved by NaCl will be the same as or better than freshly made LBP - for 12 months.

Experiment Series 3 studied the effect of LBPs of different storage durations on spring wheat germination. Higher sodium chloride concentrations (5% to 10%) and longer storage durations (3 months to 10 months) of LBPs were the clearest parameters to demonstrate the negative effect of the preservative on the spring wheat seeds. The latter acquired a lower germinating ability, poorer biological indicators of their seedlings and root system, higher percentage of affected seeds. This is obviously caused by the inhibiting effect of chloride ions of the preservative. This paper shows just a part of our experimental results obtained, namely those involving LBP with 5% NaCl in the spring wheat seeds germination throughout the whole storage period of LBP (Table 7).

Table 7. Effect of LBP with preservative (5% NaCl) on the spring wheat seeds germination during up to 12 months

Indicators	Seed germination in water	Seed germination in fresh LBP	Seed germination in LBP + 5% NaCl after:			
			3 months	6 months	9 months	12 months
Average germination,%	96.0	98.7	98.3	97.3	94.7	94.0
Average length of sprouts/root system, cm	12.2/13.1	16.7/16.9	15.9/15.1	14.6/14.5	13.1/12.9	12.4/12.8
Average mass of sprouts/root system, mg of absolutely dry substance	8.26/5.63	10.12/7.66	9.39/7.57	9.10/7.50	8.94/6.97	8.12/6.22

Description: As seen from Table 7, the properties of the preserved LBP deteriorate with its storage duration. Nevertheless, all indicators tested in seed germination by LBP stored no longer than six months, though were poorer than in freshly made LBP but exceeded substantially those in using ordinary water. This experiment showed that LBP storage with preservatives longer than six months is economically unreasonable.

4. Conclusions

As a result of the work, the following conclusions can be drawn:

1) LBP requires a stabilizer for a long-time storage;

2) 5% sodium chloride is recommended as a stabilizer - provided that LBP is stored at a low temperature (in a refrigerator or an unheated cellar);

3) LBP containing the preservative should not be stored longer than six months – only this case ensures unaffected LBP properties and a good application effect.

It should be noted that the method for LBP production was patented. However, it is planned to study further the effect of other stabilizer types and/or their mixes on the quality of LBP. Finally, it will allow improving the technology for storage of the new LBP developed for crop production and agriculture. We believe that the results of this work can be also used by manufacturers of similar biological products.

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