Bio-gel enzymes

The waste products of the bacteria contained in the bio-gels include, first and foremost, enzymes (from the Latin word “fermentum” – ferment) or, as they are also called, enzymes (from the Greek word “enzyme” – ferment).

Bacteria digest food after it is processed by the enzymes produced by the bacteria. Enzymes can increase reaction rate by up to 10^{10} times. Practically it means that the reaction duration can be reduced from 300 years to 1 second. Converting unavailable compounds contained in the soil into those available for bacteria is performed by a certain enzyme. For example, the breakdown of the cellulose contained in the soil in the form of straw residues, plant roots is performed by cellulose enzymes: gluconases, glycosidases. Soil carbohydrate residues are broken down by glycosidase enzymes.

Speaking of the enzymatic activity of biological preparations, primarily these two types of enzymatic activity are meant.

Considerable activity of bio-gels made with certain technological "nuances" has been proved experimentally. In particular, sufficiently high catalase and glucose oxidase bio-gel activity has been proven. This gel is classified as “Fungi-gel” and used for improving yield quality and soil fertility, in particular, for increasing the amount of available phosphorus. This "Fungi-gel" also has a fairly pronounced cellulase activity, that is, the ability to process plant soil residues after harvest. This makes it possible to classify it as an effective "stubble destructor" and a humus-maker improving soil structure.

Extract from the report

by the Institute of Microbiology and Virology named after A.D. Zabolotny, National Academy of Sciences, Ukraine

Biological gel (“Bio-gel”®) were studied in the period of the summer and autumn, 2016 under the direction of L.D. Varbanets, Doctor of Biological Sciences, as part of the Cooperation agreement.

Studies of “Bio-gel” cellulase activity

Cellulosolythic complex is a group of enzymes capable of performing the degradation of vegetable raw materials and, in particular, that of the most common biopolymer – cellulose. The enzymes hydrolyzing cellulose and hemicellulose include endo- and exoglucanase, β-glucosidase, mannanase,

xylanase. The composition and activity of the individual components of the complex can vary considerably depending on the composition of the substrate and environmental conditions.

Methods of determining the activity

1. ***Studies of β-glucosidase activity using synthetic nitrophenyl substrate***

Reagents:

1. Substrate: *n*-nitrophenyl-β-D-glucopyranoside ("Sigma-Aldrich", USA)
2. Phosphate-citrate buffer (PCB), 0.1 M, pH 5.2
3. Sodium carbonate (Na_2CO_3), 1 M.

To determine the glycosidase activity two "Bio-gel" samples (2 and 3) were centrifuged and the supernatant was used to determine enzyme activity. Samples 0, 1, 4 were diluted with distilled water (1: 2), then centrifuged and the supernatant was used to determine enzyme activity. For this, 0.1 ml of the supernatant was added to 0.2 ml PCB (phosphate-citrate buffer) and to 0.1 ml 0.01 M solution of the appropriate substrate. The reaction mixture was incubated for 10 min at 37 °C. The reaction was stopped by adding 2 ml of 1 M sodium carbonate. The same components were entered into the control but in reverse order. The amount of *n*-nitrophenol chipped off during the reaction, equivalent to the amount of glucose, was determined by a colorimetric method at 400 nm. A unit of enzyme activity is its amount which hydrolyzes 1 micromole of substrate per 1 min under the experimental conditions.

2. ***Studies of cellulase activity using the dinitrosalicylic method***

Reagents:

1. Dinitrosalicylic reagent
2. Phosphate-citrate buffer (PCB), 0.1 M, pH 5.2
3. Substrates: microcrystalline cellulose, servacel, DEAE-cellulose, powdered cellulose (1%), guar gum (1%).

The reaction mixture contained 0.5 ml preparation of the appropriate concentration and 0.5 ml substrate. Incubation was carried out at 37°C for 20 minutes. Then 0.5 ml dinitrosalicylic reagent were added, the mixture was held in a boiling water bath for 10 minutes, cooled in cold water, then 15 ml distilled

water were added. The concentration of reducing substances in the sample is measured spectrophotometrically at 500 and 540 nm.

Cellulase activity in 5 "Bio-gel" samples is not found. Hydrolysis zones were not identified as "Bio-gel" and supernatant were applied to agar medium containing carboxymethyl cellulose.

At the same time studies were made of cellulolytic activity of six microorganisms cultures isolated from "Bio-gel". Microorganisms were cultivated in beef-extract broth and synthetic medium, 1% carboxymethyl cellulose being used as carbon source. After incubation for three days enzymatic activity was determined in the supernatant culture liquid.

Cellulase activity of the culture liquid of six unidentified microorganisms isolated from "Bio-gel"

Culture	Enzymatic activity											
	Cellulase activity (on the following substrates)								β-glucosidase		β-mannanase	
	CM-cellulose		MC-cellulose		DEAE-cellulose		Powered cellulose					
	OD (500 nm	U/ml	OD (500 nm	U/ml	OD (500 nm	U/ml	OD (500 nm	U/ml	OD (400 nm	U/ml	OD (540 nm	U/ml
1.1	0.04	0.5	0.12	1.56	0.02	0.26	0	-	0	-	0	-
1.2	0.08	**1.01**	0	-	0.04	0.5	0.02	0.26	0	-	0	-
2	0.025	0.32	0.16	**2.04**	0.02	0.26	0	-	0	-	0	-
3.1	0.1	**1.27**	0.07	0.9	0.02	0.26	0.04	0.5	0	-	0	-
3.2	0.04	0.5	0.065	0.83	0.03	0.38	0	-	0	-	0	-
4	0.02	0.26	0.31	**1.9**	0.01	0.12	0	-	0	-	0	-

" – " no activity

To determine protein total content the Lowry method was used, to determine the total carbohydrate content the phenol-sulfuric method was used.

Total ***content of soluble protein and carbohydrates in "Bio-gel" (%)***

	Sample 0	Sample 1	Sample 2	Sample 3	Sample 4
Carbohydrate	1.04	0.35	0.31	0.007	0.8
Protein	0.16	0.05	0.02	0.004	0.19

Determining antagonistic activity in cultures 1.1, 1.2, 2, 3.1, 3.2, 4 isolated from "Bio-gel" № 1, 2, 3, 4).

Petri dishes with solid nutrient medium (beef-extract agar), test-cultures *Escherichia coli* 2884 and *Pantoea agglomerans* 8674 were used in the experiment.

Test cultures were streaked along the dish, the cultures under research were seeded perpendicular to them. Incubation was performed at 28°C for 2 days. The analysis of the results showed no test cultures growth inhibition by the strains isolated from “Bio-gel”.