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INFLUENCE OF DETERGENT AND SOME COMPONENTS OF DETERGENT ON BIOPRODUCTION OF ORGANIC ACIDS AND ENZYMATIC ACTIVITY OF FUNGI

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The fungi Aspergillus niger and Trichoderma viride were grown on the nutrient medium according to Czapek with or without washing powder detergent ethoxyl oleyl-cetyl alcohol or sodium tripoly phosphate at 0,5% concentrations. The production of free and total organic acids and the proteolytic enzymic activity were determined after 4, 7, 10, 14, and 19 days incubation.

The proteolytic enzymic activity of the fungi A. niger and T. viride was stimulated with detergent and sodium tripoly phosphate at 0,5% in the medium during aging of the cultures.

Key words: Aspergillus niger, detergent, ethoxyl oleyl-cetyl alcohol, proteolytic activity, sodium tripoly-phosphate, Trichoderma viride

INTRODUCTION

Detergents appear as significant pollutants in the environment (Clark, 1966), particularly of water ecosystems. With the increase of the standard of living, the use of detergents significantly increases both in households and industry (Ljubisavljević, 1985). Therefore there is a need for purification that is, removal of detergents from the environment, where a significant role is played by microorganisms such as fungi (Stojanović, 1988, Gilmore *et al.*, 1994). Some tensides such as the linear tenside, acryl-benzene sulfate are very easily decomposed by microorganisms.

It was shown that a detergent powder and its oleyl-cetyl alcohol and sodium tripoly- phosphate influence the bioproduction of organic substances (free and total organic acids), (Stojanović, *et al*, 1986), and also affect the enzymatic activity of the species *A. niger, T. roseum* and others (Stojanović, *et al.*, 1994).

MATERIALS AND METHODS

The experiments were performed in the Laboratory for Biochemistry of the Faculty of Science in Kragujevac using monosporial cultures of the fungi *Aspergillus niger* van Tiegheme and *Trichoderma viride* Pers ex. S. F. Gray isolated from the river Lepenica where waste water flaw. These species of fungi were chosen because they were most abundant there. The monospore cultures of the

fungi were obtained by the methods of exhaustion on a poor potato-dextrose agar (Gorlenko, *et al.*, 1976).

The fungi were then grown in the liquid nutrient medium according to Czapek, of standard composition. The detergent "Merix" (Merima, Kruševac) designated D; ethoxyled oleyl-cetyl alcohol (AOC) and sodium tripoly-phosphate-(TTP) were added at 0,5% concentrat and the flasks incubated for 19 days. The flasks containing 200 ml of nutrient medium were shaken ona a Kinetor shaker at 250 revolutions a minute at room temperature (22°C) in conditions of alternate light - dark cycles (day-night).

Spores of the fungi in question were suspended in 5 ml of sterile water and density was checked by hemocytometer for the erythrocytes. One drop of each suspension was added to a serial of flasks and changes followed at 4, 7, 10, 14 and 19 days from the day of inoculation. The pH value and the redox potential (rH_2) were measured with the pH-meter of the type MA-5-105 (Iskra Kranj) using the formula: rH_2 E / 0,029 2pH, where is the E-electrical potential in mV. The proteolytic activity was determined by the Anson method (Dudka, 1982) from the amount of tyrosine and tryptophan, produced by hydrolysis of casein by proteolytic enzymes in 1 ml medium filtrate (Petrović, *et al.*, 1971) The proteolytical activity was expressed in arbitrary units PE according to the following formula:

$$PE = (a-b)8p / 181x10$$

PE- proteolytic unit;

a - mg- tyrosine in experimental solution

b - mg- tyrosine found in filtered casein alone;

181 - molecular mass of tyrosine;

10 - time of duration of enzymatic reaction in minutes;

p - dilution of filtered liquid.

Free and total organic acids were separated by passage through activated cation columns (Amberlite - IR - 120), (Veličković,1971).

For the determination of free organic acids 10 ml of medium was taken and mixed with 50 ml of ethanol. After incubation at 70°C in a water bath for 1-1,5 hours, the mixture was filtered through a special filter. The filtrate was at 50-60°C at reduced pressure to the volume of 40 ml transferred to a volumetric flask and made up to 100 ml after addition of a teaspoon of the active charcoal. After standing in a water bath for 30-45 min at 70°C, 10 ml aliquts of filtrate were taken for the determination of the free organic acids by titration with 0,1 M NaOH (Veličković, 1971).

Employing hypothesis that mean 1 (H0) is equal to mean 2 (H1), Student's t-test was used to detect significant differences between data obatined for the control series and data for the treated series. The results were first tested for equal variance using the F-test. The hypothesis was rejected when =0,05 and =0,01 (Milošević, 1982).

RESULTS AND DISCUSSION

The influence of "Merix" detergent, and its components AOC and TTP on the proteolytical activity of the fungal species *A. niger* and *T. viride* 4, 7, 10, 14 and 19 days after inoculation is shown in Figures 1. and 2.

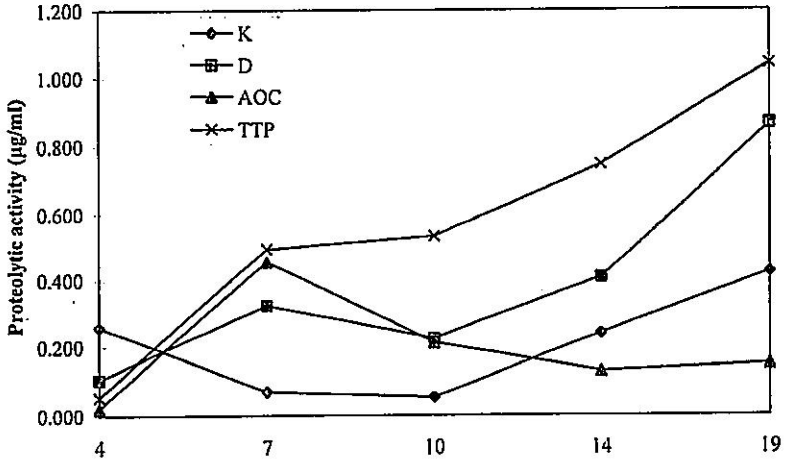

Figure 1. Proteolytic activity of *A. niger* incubated in the nutrient base of Czapek without (K) and with detergent (D) or its components (AOC, TTP) at 0,5% concentration

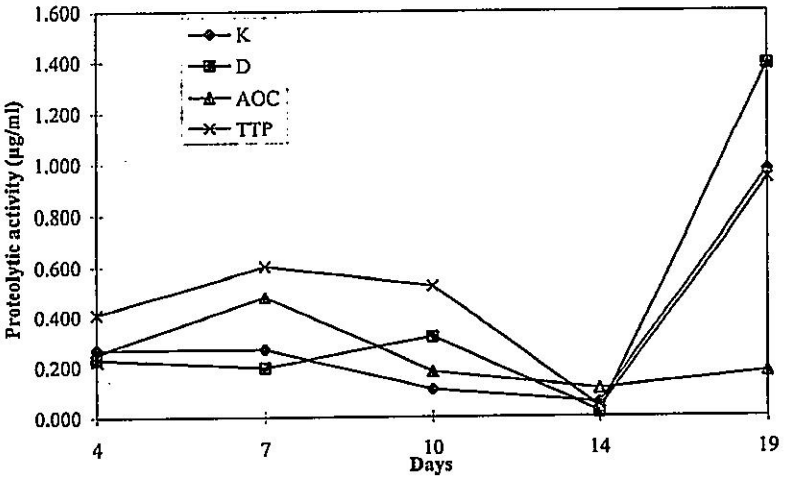

Figure 2. Proteolytcc activity of T. viride incubated in the nutritent base of Czapek Czapek without (K) and with detergent (D) or its components (AOC, TTP) at 0,5% concentration

A. niger grew in all the different nutrient media and showed very intensive proteolytical activity, which increased with the age of the culture in the medium with added TTP and whole detergent (Stojanović *et al.*, 1994).

However, the difference in mean values for proteolytical activity in the observed media was not statistically at the probability levels of 0,05 and 0,01 (Milošević,1982). for any treatment in comparison with control values.

Although *T. viride,* grew in all the nutrient media proteolytical activity was somewhat weaker than for *A. niger* especially up to 14 day culture. With AOC weak proteolytical activity occurred at the end of the experiment with both fungi (Egorova, 1976).

For *T. viride* introducing AOC at 0,5% concentration into the medium led to a statistically significant, change of proteolytical activity in comparison with the control medium at the threshold of probability of 0,05 and 0,01.

Differences between the mean values for the proteolytical activities of the control medium and the media with whole detergent or TTP at 0,5% concentration were not statistically significant.

The species of *A. niger* and *T. viride* grown in all variants of nutrient media produced free and total organic acids (Veličković, 1971, Stojanović, 1989) The production of the total organic acids was significantly greater than the production of free organic acids (Table 1).

Table 1. The change of concentration of organic acids (free and total, expressed as %) in the nutrient medium according to Czapek during incubation of fungi without (K), with detergent (D), or its components (AOC, TTP) at 0,5% concentation for 4-19 days

Culture	Free organic acids					Total organic acids				
		D	a	y	s		D	a	y	s
	4	7	10	14	19	4	7	10	14	19
Aspergillus niger (K)	1.80	2.50	4.00	2.20	3.48	2.00	2.80	4.20	4.00	4.50
Aspergillus niger (D)	1.80	1.80	4.40	2.80	3.60	2.60	2.20	4.50	4.00	5.00
Aspergillus niger (AOC)	0.50	0.50	2.00	1.00	2.24	1.60	0.72	2.20	2.00	2.50
Aspergillus niger (TTP)	1.20	1.00	2.20	2.00	2.40	1.88	1.60	2.40	3.20	2.90
Trchoderma viride (K)	2.60	0.40	1.60	0.60	1.00	3.50	1.50	3.00	1.50	1.20
Trchoderma viride (D)	1.00	1.20	0.40	0.40	1.00	1.50	2.00	3.00	1.00	1.60
Trchoderma viride (AOC)	1.80	1.20	0.40	2.60	1.00	3.50	2.50	3.00	5.00	1.50
Trchoderma viride (TTP)	1.00	1.60	1.00	0.80	1.40	2.00	2.00	1.00	1.50	2.00

A. niger produced significantly more free and total organic acids than *T. viride.* The production of free acid increased with the age of culture of *A. niger* and tended to decline with *T.viride.*

There were statistically significant differences in free and total organic acids in the medium with 0,5% TTP compared with the control (Džamić and Veličković, 1970, Stojanović, 1989). This was not the case with other pollutants at the applied concentration. (Moore *et al.* 1958, Narayanan *et al.* 1966, Stojanović, *et al.*, 1994).

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UTICAJ DETERDŽENTA I NEKIH KOMPONENTI DETERDŽENTA NA BIOPRODUKCIJU ORGANSKIH KISELINA I ENZIMSKU AKTIVNOST GLJIVA

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja uticaja deterdženta "Merix" (Merima, Kruševac) i komponenti deterd'enta (etoksil oleil-cetil alkohola i natrijum tripolifosfata) u koncentraciji od 0,5% na produkciju slobodnih i ukupnih organskih kiselina i enzimsku aktivnost vrsta gljica *Aspergillus niger* i *Trichoderma viride*

Rezultati ovih ispitivanja ukazuju da *A. niger* produkuje znatno više organskih kiselina, posebno ukupnih, u odnosu na *T. viride* i to u hranljivoj podlozi sa deterdžentom u koncentraciji od 0,5%. U svim hranljivim podlogama *A. niger* ispoljava veoma značajnu proteolitičku aktivnost. Ona je bila veoma izražena u starijim kulturama posebno u podlogama sa natrijum tripoli fosfatom u koncentraciji od 0,5% a nešto manja u podlogama sa samim deterd'entom.