Acta Veterinaria (Beograd), Vol. 60, No 1, 67-77, 2010.

DOI: 10.2298/AVB1001067S UDK 619:579.62

THE INFLUENCE OF DETERGENTS, SODIUM TRIPOLY-PHOSPHATES AND ETHOXYLED OLEYL-CETYL ALCOHOL ON METABOLISM OF THE FUNGI *PENICILLIUM VERRUCOSUM* PEYRONEL

STOJANOVIĆ JELICA*, JAKOVLJEVIĆ VIOLETA*, MATOVIĆ IVANA*, MIJUŠKOVIĆ Z** and NEDELJKOVIĆ T***

**Faculty of Natural Science and Mathematics, University of Kragujevac, Serbia*
***Institute for Medical Biochemistry Military Medical Academy, Belgrade, Serbia*
****Medical Faculty Kragujevac, Serbia*

(Received 8th June 2009)

On the species of the fungi Penicillium verrucosum grown on liquid nutritious base, according to Czapek and on a variation of the same nutritious base with detergent MERIX ("Merima", Kruševac) and individual components of the same detergent: sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1%, the following analyses were performed: pH, redox potential, proteolytic activity, the quantity of free and total organic acids, amino acids, proteins and total biomass.

We can notice from the obtained results that the detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% had an inhibitory effect on protein bioproduction and total biomass on the 8th day from the day of inoculation, while the production of free and total organic acids was partially or significantly stimulated by the presence of the detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1%. With aging of the fungus culture Penicillium verrucosum, the exception was free organic acids production which was, with the age of the culture, partially inhibited by the detergent. Proteolytic activity of the fungi Penicillium verrucosum had a slightly variable value during the experimental period with added nutritions media to the detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% with a slight aberration in relation to the control in some phases of the fungal growth.

Key words: amino acids, biomass, pH, proteins, redox potential

INTRODUCTION

Microorganisms, first of all bacteria and fungi, have the ability of biodegradation of organic compounds, which come to the living environment by the activity of man. Among the fungi that have this ability the filamentous fungi from the group *Deuteromycetes* or *Imperfect fungi* are especially separated, for

the sake of their physiological, morphological and biochemical characteristics (Raimbault, 1981).

The apical growth of the hypha tip enables the penetration into solid substrates and the secretion of extracellular enzymes from vesiculae, which are placed on the tip of the hypha into the environment. Under the effect of these enzymes, the complex organic compounds are decomposed into simpler ones which can be used by the fungus for its growth and development of the mycelium and so they are built into the biomass (Raimbault, 1998; Saucedo-Castañeda *et al*., 1992a; 1992b).

Among 1500 various pollutants found in the living environment, first of all in water ecosystems and in the soil, detergents play an important role in regard to the extent of their production and usage.

Nowadays detergents of various compositions can be found on the market' but all of them can be divided into two groups: phosphate detergents and detergents with the surface active agent.

Phosphates detergents are highly caustic, they modify pH media, induce eutrophication (the blooming of algae) in fresh waters, toxin release and decreased dissolved oxygen.

Detergents with the surface active agent if they are present in significant quantities can have toxical effects on all species of aquatic living creatures, including biodegradable detergents (Moreno *et al*., 1990).

The linear alkyl-benzene sulphonated (LAS) is the anion surface active agent used almost only in detergents for more than 30 years. LAS is very easily degraded under the activity of microorganisms in waste water plants (Waters and Feijtel, 1995; Holt *et al*., 2003). The primary LAS biodegradation on the activated sludge is higher than 99% (EU Commission, 1997). Traces of LAS in natural waters (Itrich and Federle, 1995) and in the soil (Küchler and Schnaak, 1997) continue the quick biodegradation (half life of LAS is about 0.15 - 0.5 days). The ultimate biodegradation of LAS quantity is 95-98 % so that they are not harmful for the environment and living creatures.

By analysis of the activated sludge microbiological community in waste water plants and waste waters passing through populated places a great number of different fungi species degrading detergents as in aerobic so in anaerobic conditions were identified (Sanz *et al*., 2003).

MATERIAL AND METHODS

A monosporial culture of the fungi species *Penicillium verrucosum* Peyronel was isolated and determined from the river basin of Lepenica (the place of waste water flood, sewage). When deciding which species of fungi to use, the quantitative representation of the fungi species in a precisely determined volume of the water sample was taken into consideration. The isolated and determined fungi species were maintained in a chamber with a constant temperature of 4^{o}C (±0.5^{o}C), on potato-dextrose agar slant, in sterile conditions.

A monospore culture was developed by the method of exhaustion on a poor agar, in Petri dishes, under sterile conditions. Mesopeptonic agar was used for sterility control.

During the experiment, the fungi were grown on the sterile nutrient base of the following composition: $NaNO_3$ - 3, K_2HPO_4 - 1, $MgSO_4$ - 1, $MgSO_4x7H_2O$ - 0.25, $FeSO_4x7H_2O$ - 0.01, saccharose - 30, distilled water - 1000 mL (control - K) and on a variation of the liquid nutritious base, according to Czapek, with detergent MERIX ("Merima", Kruševac, mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in a concentration of 0.1%. The variant of the liquid nutritious base was stored in Erlenmeyer bottles (200 mL of base in 250 mL bottles). Erlenmeyer bottles were placed on an electric shaker, thus enabling uniform and constant mixing (aeration of the fungi).

The experiments were carried out at room temperature, under alternate light and dark cycles (Stojanović, 1990).

The following analysis of the fungi *Penicillium verrucosum* grown on a liquid nutritious base, according to Czapek (control) and on the variation of the liquid nutritious base, with added detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1%, were observed: pH reaction of the medium, redox potential, proteolytic activity, the amount of proteins on the 4th, 5th, 6th, 7th and 8th day, the amount of free and total organic acids on the 4th and 8th day. The quantitative and qualitative composition of amino acids and the quantity of total biomass were determined on the 8th day.

"Iskra", Kranj, MA-5705 pH meter was used for measuring of the pH reactions of the medium.

The redox potential (rH_2) has been determined according to the formula:

$$rH_2 = E/0{,}029 + 2pH,$$ E – the electrical potential given in mV.

The degree of the fungal proteolytic activity was determined by the Anson method (Dudka, 1982; Egorova, 1976; Petrović and Petrović, 1971) in 1 mL of the liquid nutritious base without filtration, on the basis of the amount of tyrosine and tryptophane produced by hydrolysis of casein caused by the proteolytic enzyme of the mentioned fungi.

The proteolytic activity of the fermented liquid was expressed in arbitrary units "PE" according to the formula:

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

PE – proteolytic unit
a – mg-tyrosine in the experimental solution
b – mg-tyrosine in filtered liquid casein
8 – calculated coefficient of the amount of tyrosine in relation to solution capacity
p – dilution of filtered liquid
181– molecular mass of tyrosine
10 – duration of the enzymatic reaction in minutes.

Free organic acids are determined by proportion and by acidimetric titration with 0.1 M NaOH added phenolphthalein indicator.

Total organic acids of the specimen are isolated by the ion-exchange columns (cation, Aberlite-R-120 type), their quantity were determined according the same procedure as for the free organic acids (Veličković, 1971).

The quantity of biomass of the fungi species *Penicillium verrucosum* was determined on the basis of the mass difference between dry filter paper and the total mass with fungus mycelium. The quantity of biomass is expressed in grams (g) (Stojanović *et al*., 1986).

The qualitative and quantitative determination of amino acids was performed by the standard method by means of an aminoanalyser (Egorova, 1976; Moore *et al*., 1958; Petrović and Petrović, 1971; Stein and Moore, 1954).

The quantity of proteins of the mentioned fungi species was obtained through the Kjeldahl method on the basis of nitrogen amount present in the fungus tissue (Petrović and Petrović, 1971; Laukevics *et al*., 1984), according to the formula:

Quantity of proteins = 6.25 x quantity of nitrogen (in mg).

RESULTS AND DISCUSSION

The influence of detergent MERIX ("Merima", Kruševac) and its components (sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol) in a concentration of 0.1%, on the fungi species *Penicillium verrucosum* has been observed through the following parameters: pH, redox potentials, proteolytic activity, bioproduction of free and total organic acids, amino acids, quantity of proteins and total biomass, as presented in Figures 1-7.

The fungus *Penicillium verrucosum* grown on the liquid nutritious base, (according to Czapek) and on the variant of nutritious base with detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1%, during the experimental period has shown an almost standardized pH reaction of the medium with a tendency to decrease, as presented in Figure 1.

The significant exception in relation to the control was noticed on nutritious base with detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol of the applied concentration during the period of 4-8 days, the distinct exception was noticed on growth and development of the fungus (Stojanović, 1990).

The redox potential of the fungus *Penicillium verrucosum* grown on the liquid nutritious base, (according to Czapek) and on the variant of the nutritious base with detergent added sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1%, during the experimental period had the tendency of growth, as presented in Figure 2.

The weakest tendency of the redox potential growth, the fungus achieved on the nutritious base with detergent in a concentration of 0.1%. This was the most significant exception in relation to the control (Stojanović, 1990; Veličković, 1971).

The fungus *Penicillium verrucosum* grown on the media with sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol had a slightly variable redox potential during the experimental period with exception in relation to the control in some

phases of the fungus development. With aging of the fungus, the redox potential was almost equal on the 8th day.

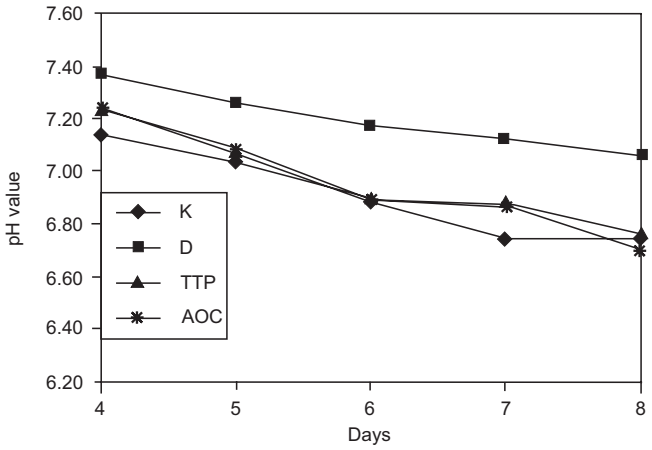

Figure 1. pH value of the *P. verrucosum* grown on the nutritious base according to Czapek (control-K) and the variant of nutritious base with detergent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 0.1% during the period of 4-8 days

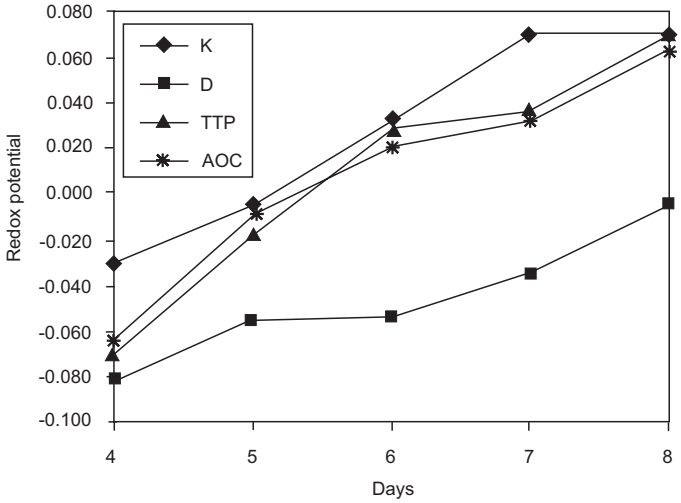

Figure 2. The redox potential of *P. verrucosum* grown on the nutritious base according to Czapek (control-K) and the variant of nutritious base with deterdgent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 0.1% during the period of 4-8 days

Species of fungi *Penicillium verrucosum* grown on the liquid nutritive media (according to Czapek) and on the same nutritious base with added detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% achieved enzyme activities during the experimental period of 4 to 8 days, with a slight variability during the period of 5-7 days, as presented in Figure 3.

The fungus achieved the enzymatic activity maximum on the 6th day on the nutritive media with added sodium tripoly-phosphate, and on the 7th day on the media with added detergent, ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% and in the control (Stojanović, 1990; Stojanović *et al.*, 2002; Saucedo-Castañeda *et al.*, 1992a).

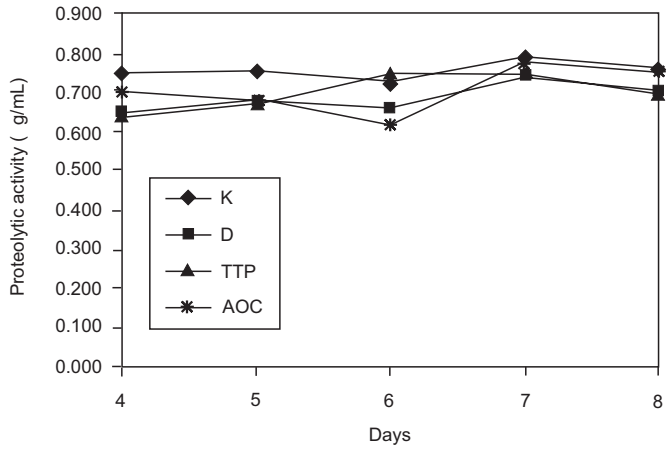

Figure 3. Changes of the proteolytic activity of *P. verrucosum* grown on the nutritious base according to Czapek (control-K) and the variant of the nutritious base with detergent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC)in concentration of 0.1% during the period of 4-8 days

The enzyme activity of the fungus *Penicillium verrucosum* grown on the media with added sodium tripoly-phosphate and ehtoxyled oleyl-cetyl alcohol in a concentration of 0.1% had a mild exception in relation to the control, and distinct exception was present until the 6th day from the day of inoculation of the mentioned species of fungus.

The bioproduction of the free and total organic acids of the fungus *Penicillium verrucosum* grown on the nutritive culture media and on the same media with added sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% was different, as presented in Figure 4.

The bioproduction of the total organic acids on the nutritious base with added detergents, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol was considerably higher than the production of free organic acids, the exception was the control base in which the production of free organic acids was

considerably higher than total organic acids on the 8th day (Veličković, 1971; Stojanović, 1990; Stojanović *et al*., 2001).

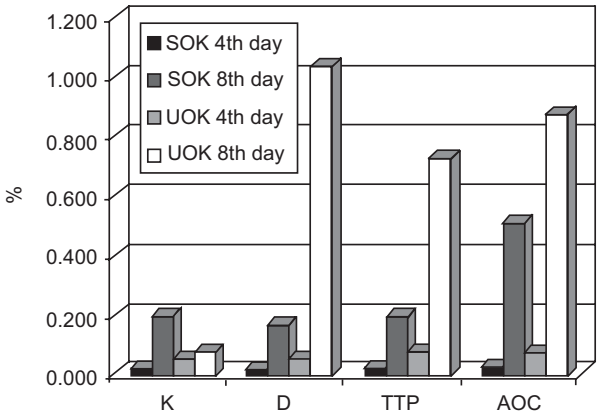

Figure 4. The quantity of free (mark SOK) and total (mark UOK) organic acids (in percentage) of *P. verrucosum* grown on the nutritious base according to Czapek (control-K) and the variant of the nutritious base with detergent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 0.1% on the 4th and the 8th day

The greatest production of total organic acids the fungus achieved on the media with added detergent in a concentration of 0.1%. Values were considerably higher in relation to the control on the 8th day.

The fungus *Penicillium verrucosum* grown on the liquid nutritive media (according to Czapek) and on the same media with detergent added in a concentration of 1% produced 15 different amino acids, as presented in Figure 5.

The detergent in a concentration of 1% significantly inhibited the production of 13 different amino acids, and the highest effect was on asparagine and glutamic acid, tyrosine and phenylalanine. The same pollutant of the applied concentration stimulated the production of the amino acids isoleucine and lysine (Stojanović, 1990; Stojanović *et al*., 2002; Stein and Moore, 1954).

The bioproduction of the proteins of the fungus *Penicillium verrucosum* grown on all of the mentioned variations of the media was weak and insignificant until the 6th day. From the 6th to the 8th day, the production of this matter significantly increased in the fungus *Penicillium verrucosum* grown on the liquid media, while the production of this matter on the media with detergent added in a concentration of 0.1% had the tendency of growth from the 7th to the 8th day, but significantly less in relation to the control (Raimbault, 1981; Waters and Feijtel, 1995). On the nutritious base with sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% the bioproduction of proteins was very weak and had the tendency to decrease from the 7th to the 8th day, as presented in Figure 6.

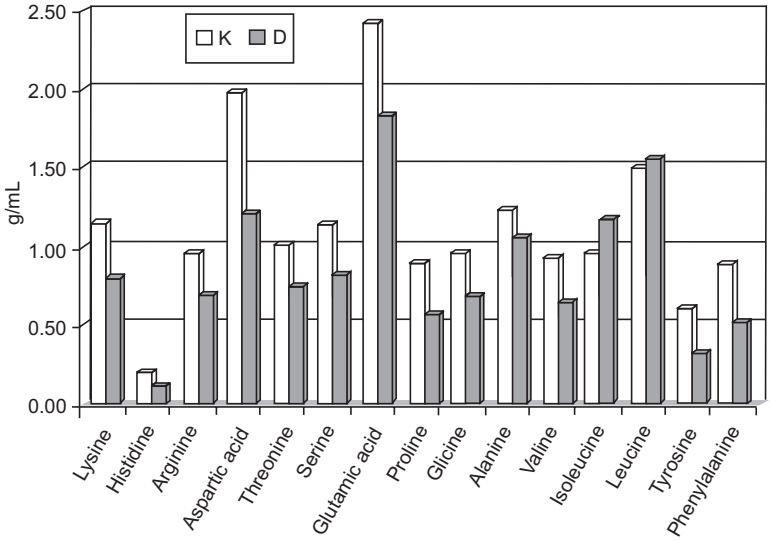

Figure 5. The qualitative and quantitative composition of amino acids (g/mL) of *P. verrucosum* grown on the nutritious base according to Czapek (controla-K) and the variant of nutritious base with detergent (mark-D) in concentration of 0.1% koncentracije on the 8th day

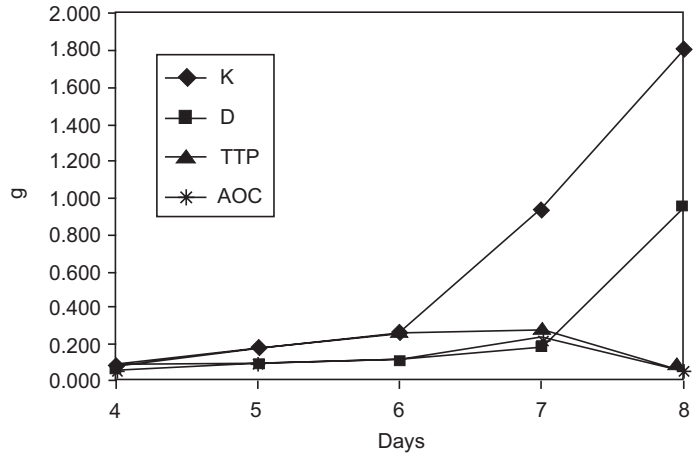

Figure 6. The quantity of proteins (in grams) of *P. verrucosum* grown on the nutritious base according to Czapek (control-K) and the variant of nutritious base with detergent (mark D), sodium tripoly-phosphate (mark TTP) and ethoxyled oleyl-cetyl alcohol (mark AOC) in concentration of 0.1% during the period of 4-8 days

The total biomass of the fungus *Penicillium verrucosum* grown on the liquid nutritious base, according to Czapek (control) and on the nutritious base with

added detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% was variable depending on the nutritious base type, as presented in Figure 7.

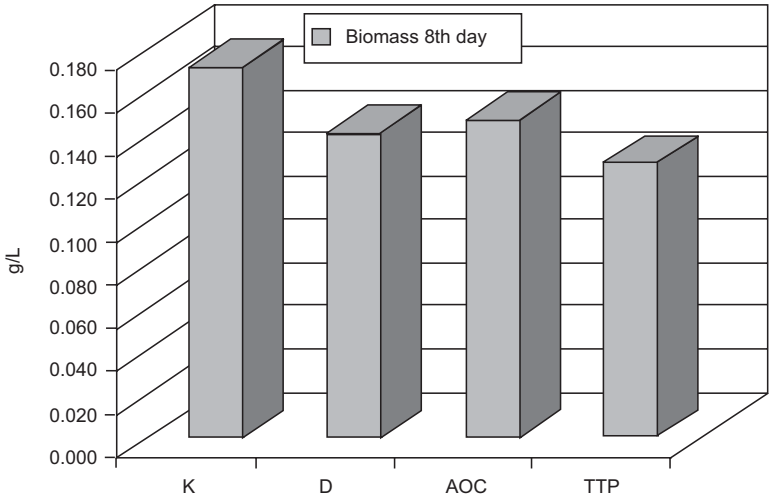

Figure 7. The quantity of biomass (g/L) of *P. verrucosum* grown on the nutritious base according to Czapek (control-K) and the variant of nutritious base with detegent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 0.1% on the 8th day

Detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in a concentration of 0.1% inhibited the total biomass bioproduction of the fungus *Penicillium verrucosum* in different ways: sodium tripoly-phosphate as the most significant, and then detergent, while ethoxyled oleyl-cetyl alcohol showed the weakest inhibitory effect in relation to the control (Waters and Feijtel, 1995).

On the basis of the obtained results it can be concluded that the detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol added in the liquid nutritious base, according to Czapek (control), in a concentration of 0.1% have the ability to change the pH medium (with the tendency of decreasing), so that increasing the redox potential, the production of total and free amino acids, but decreasing the growth and development of the fungal species.

Address for correspondence:
Dr Jelica Stojanović
Faculty of Natural Science and Mathematics
Kragujevac, Serbia
E-mail: jelica@kg.ac.rs

REFERENCES

1. *Dudka A*, 1982, Methods of Experimental Ecology (in Russian), Naukova Dumka, Kiev.
2. *Egorova NC*, 1976, Microbiology Workbook (in Russian), Moscow University, Moscow, 244.
3. EU Commission DGIII, 1997, Study on the possible problems for the aquatic environment related to surfactants in detergents, WRC, EC 4294.
4. *Holt MS, Fox KK, Daniel M, Buckland H*, 2003, LAS and Boron monitoring in four catchments in the UK contribution to GREAT-ER#11, *Sci Total Environ,* 314-316, 271-88.
5. *Itrich NR, Federle TW*, 1995, Primary and ultimate biodegradation of anionic surfactants under realistic discharge conditions in river water, *SETAC Meeting*, Vancouver, Canada.
6. *Küchler T, Schnaak W*, 1997, Behavior of LAS in sandy soils with low amounts of organic matter, *Chemosphere,* 35, 153-67.
7. *Laukevics JJ, Apsite AF, Viesturs UE, Tengerdy RP*, 1984, Solid substrate fermentation of wheat straw to fungal protein, *Biotechnol Bioeng*, 26, 1465-74.
8. *Moore S, Spackman D, Stein W*, 1958, Chromatography of aminoacids on sulphonated polystyrene resins, *Anal chem*, 30, 7, 1185-90.
9. *Moreno A, Ferrer J, Berna JL*, 1990, Biodegradability of LAS in a Sewer System, *Tenside Surf Det*, 27, 312-5.
10. *Petrović S, Petrović J*, 1971, Priručnik iz biohemije, BIGZ, Beograd.
11. *Raimbault M*, 1981, Fermentation en milieu solide: croissance de champignons filamenteux sur substrats amylacés, Edited by: ORSTOM-Paris; Série Travaux et Documents n^{o} 127, 291.
12. *Raimbault M*, 1998, General and microbiological aspects of solid substrate fermentation, *Electron J Biotechnol,* URL: http://www.scielo.cl/scielo.php?pid=S0717-34581998000300007&script=sci_arttext
13. *Sanz JL, Culubret E, de Ferrer J, Moreno A, Berna JL*, 2003, Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in upflow anaerobic sludge blanket (UASB) reactors, Biodegradation, 14, 1, 57-64.
14. *Saucedo-Castañeda G, Lonsane BK, Navarro JM, Roussos S, Raimbault M,* 1992a, Potential of using a simple fermenter for biomass built up, starch hydrolysis and ethanol production: Solid state fermentation system involving *Schwanniomyces castellii*, *Appl Biochem Biotechnol*, 36, 47-61.
15. *Saucedo-Castañeda G, Lonsane BK, Krishnaoih MM, Navarro JM, Roussos S, Raimbault M,* 1992b, Maintenance of heat and water balances as a scale-up criterion for the production *of ethanol by Schwanniomyces castellii* in a solid state fermentation system, *Process Biochem*, 27, 97-107.
16. *Stein WH, Moore S*, 1954, The free aminoacids of human blood plasma, *J Biol Chem*, 211, 2, 915-26.
17. *Stojanović J, Ristanović M, Vučetić J*, 1986, Prilog poznavanju biohemijskih osobina nekih gljivica *in vitro*, *Mikrobiologija*, 23, 1, 73-9.
18. *Stojanović J*, 1990, Uticaj deterdženata na biohemijska svojstva nekih gljivica *in vitro*, Doktorska disertacija, Prirodno-matematički fakultet, Univerzitet u Kragujevcu.
19. *Stojanović J, Stojanović M, Milovanović A,* 2001, Uticaj deterdženta i nekih komponenti detedženta na bioprodukciju organskih kiselina i enzimsku aktivnost gljiva, *Acta Veterinaria (Belgrade)*, 51, 2-3, 171-6.
20. *Stojanović J, Veličković D, Vučetić J,* 2002, Uticaj deterdženta i nekih komponenti detedženta na bioprodukciju organskih materija i enzimsku aktivnost dve vrste gljiva, *Acta Veterinaria (Belgrade),* 52, 4, 267-72.
21. *Veličković D,* 1971, Prilog poznavanju dinamike aminokiselinskog i proteinskog sastava jabučastog voća u toku vegetacije i čuvanja, Doktoska disertacija, Poljoprivredni fakultet, Univerzitet u Beogradu.
22. *Waters J, Feijtel, TCJ*, 1995, AISE/CESIO environmental surfactant monitoring programme: outcome of five national pilot studies on LAS, *Chemosphere*, 30, 1939-56.

UTICAJ DETERDŽENTA, NATRIJUM TRIPOLI – FOSFATA I ETOKSILOVANOG OLEIL-CETIL ALKOHOLA NA METABOLIZAM GLJIVE *PENICILLIUM VERRUCOSUM* PEYRONEL

STOJANOVIĆ JELICA, JAKOVLJEVIĆ VIOLETA, MATOVIĆ IVANA, MIJUŠKOVIĆ Z i NEDELJKOVIĆ T

SADRŽAJ

U ovom radu su izneti rezultati uticaja deterdženta "MERIX" (Merima, Kruševac) i komponenti deterdženta (natrijum tripoli-fosfata i etoksilovanog oleil-cetil alkohola) 0,1% koncentracije na promenu: pH sredine, redoks potencijala, enzimsku aktivnost, bioprodukciju slobodnih i ukupnih organskih kiselina, proteina i ukupne biomase gljive *Penicillium verrucosum* Peyronel.

Rezultati ovih istraživanja ukazuju da gljiva *Penicillium verrucosum* produkuje znatno više ukupnih organskih kiselina i to u hranljivoj podlozi sa deterdžentom (najviše), a zatim sa etoksilovanim oleil-cetil alkoholom i natrijum tripolifosfatom u koncentraciji 0,1%, dok je produkcija slobodnih organskih kiselina izrazita u hranljivoj podlozi sa etoksilovanim oleil-cetil alkoholom iste koncentracije.

Produkcija amino kiselina (sa izuzetkom amino kiseline leucina i izoleucina), proteina i ukupna biomasa inhibirana je prisustvom deterdženta, natrijum tripolifosfata i etoksilovanog oleil-cetil alkohola u hranljivoj podlozi gljive *Penicillium verrucosum.*