Bulgarian Chemical Communications, Volume 49, Number 2, (pp. 390 – 398) 2017

Comparative study of some biochemical parameters of the fungi *Mucor plumbeus*, *Aspergillus niger* and *Trichoderma harzianum*

I. Matović-Purić[1], D. Pecarski[2], Z. Jugović[3], D. Jovičić[4], D. Đorđević[5], P. Maškoviċ[6]

[1]*Medical School, Episkopa Nikifora Maksimovića no. 8, Čačak, Serbia*
[2]*Higher Education School of Professional Health Studies, Cara Dusana 254, Belgrade, Serbia*
[3]*High-sanitarian Health School of Professional Studies "Visan", Tošin bunar 7a, Belgrade, Serbia*
[4]*Singidunum University, Faculty of Applied Ecology – Futura, Požeška 83, Belgrade, Serbia*
[5]*University of Niš, Faculty of Sciences and Mathematics, Višegradska 33, Niš, Serbia*
[6]*University of Kragujevac, Faculty of Agronomy, Čačak, Serbia*

Received September 12, 2016; Accepted October 21, 2016

The aim of this study was to examine the effect of high concentrations of the commercial detergent "Merix" (Henkel, Kruševac) on the growth, development and biochemical characteristics of the tested fungi isolated from sewage and industrial wastewater. Metabolic activity of *Mucor plumbeus*, *Aspergillus niger* and *Trichoderma harzianum* grown in such liquid nutrient medium and control medium is monitored over following biochemical parameters: amounts of free and total organic acids, pH values, redox potential and biomass. Depending on the fungus species, there was a biomass inhibition at various concentrations under the influence of the detergent in the period from the third to the sixth day. Detergent concentration of 0.3% was decomposed in all tested fungus - detergent mixtures.

Key words: *Aspergillus niger*, *Mucor plumbeus*, *Trichoderma harzianum*, detergent, ethoxyl-oleyl-cetyl alcohol, sodium tripolyphosphate

INTRODUCTION

Many researchers stated that microorganisms, particularly some kinds of fungi, can act as potential degraders of detergents [1,2]. Among the fungi which have such ability, filamentous fungi (*Deuteromicotina)* are especially distinguished due to their physiological and biochemical characteristics [5]. Specificity in apical growth of these fungi enables penetration in solid substrates and excretion of extracellular enzymes from vesicles on the top of hyphae to environment. Under the influence of these enzymes complex organic compounds are decomposed to simpler ones, thus the fungi can be used for growth and development of mycelia and biomass accumulation [3,4,6]. Filamentous fungi are attractive microorganisms for the study of biodegradation of organic matter due to their well-developed defense mechanisms and structure of the cell wall. Fungi are recognized for their superior capability to produce a large variety of extracellular proteins, organic acids and other metabolites, as a result of adaptation to severe environmental constraints [7]. *Mucor racemosus Fresenius* is a dimorphic fungus (genus Mucor) whose growth is induced by carbon dioxide and hexose sugar in the direction of creating a multipolar bud as in yeast or in the direction of branched aerial hyphens [8]. When grown on synthetic and organic substrates *M. racemosus* produces various enzymes such as invertase, alkaline phosphatase, protease, lipase, which have applications in biotechnology and bioremediation.

The *Aspergillus* fungus was first recognized as an organism in 1729 by Micheli [9]. The genus *Aspergillus* is found worldwide and consists of more than 180 officially recognized species, and comprises a particularly important group of filamentous ascomycete species [10]. Although it includes the major filamentous fungal pathogen of humans, *Aspergillus fumigatus* [11], most of the members are useful microorganisms in nature for degradation of plant polysaccharides [12], and they are important industrial microorganisms for the large-scale production of both homologous and heterologous enzymes [13-17]. Among them, *Aspergillus oryzae* and *Aspergillus niger* are on the Generally Recognized as Safe List of Food and Drug Administration in the United States [18]. *Aspergillus niger* is one of the most important microorganisms used in biotechnology [19,20] which produces many extracellular enzymes.

Trichoderma spp. are common saprophytic fungi that are interactive in soil, root and foliar environments. They are well-known biocontrol agents and also have considerable metabolic diversity [11]. They are recognizable for degradation of chitin, glucans, lignin and cellulose

* To whom all correspondence should be sent:
E-mail: agrofarmzorka@gmail.com

[12]. *Trichoderma harzianum* can degrade various organic compounds such as DDT (dichlorodiphenyltrichloroethane), dieldrin, endosulfan, PCNB (pentachlororonitrobenzene) and PCP (pentachlorophenol) [13]. Therefore, *Trichoderma* strains play an important role in the bioremediation of soil contaminated with pesticides, herbicides and insecticides. Data about the proteolytic enzyme profiles of *Trichoderma* strains revealed that the protease system of *Trichoderma* is complex containing a large set of enzymes. Some of these proteases are involved in mycoparasitic action, nematicidal activity and plant colonization. However, only a few *Trichoderma* proteases have been examined until now for their potential applicability for commercial purposes.

On inoculated fungi species grown under *in vitro* conditions, in the presence of the mentioned pollutants, the metabolic changes of bioproduction of different organic compounds in various aging steps of fungi, have been investigated. The aim of this study was to find out, among the great variety of fungal species from wastewater, these which are resistant to effects of detergent and its components. Metabolic activity of *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum* grown in such liquid nutrient medium and control medium was monitored over following biochemical parameters: amounts of free and total organic acids, pH values, redox potential and biomass.

MATERIALS AND METHODS

Isolation of Mucor racemosus Fresenius and cultivation

The fungus species was isolated from wastewater samples of the Rasina River, downstream where the industrial wastewaters of the factory Henkel, Serbia, discharge into river. Sample of wastewater was taken in late May 2010. Sample was taken in a sterile container and transferred to the microbiology laboratory where it was disposed of in a refrigerator at 4°C. Within 24h, different dilutions of the sample were transferred on Petri plates with malt agar and streptomycin to prevent bacterial growth. The plates were then maintained at room temperature for 5 days. Positive cultures were subcultured on malt agar and potato dextrose agar for the isolation of a pure, single colony for identification. The identification of the fungus *M. racemosus Fresenius* (1976) was based primarily on the macroscopic and microscopic morphology and was carried out by systematic keys. The fungus was maintained on potato-dextrose-agar (PDA) slant grown at 30°C, stored at 4±0.5°C, and subcultured monthly in sterile conditions. During the experiment, the fungus was cultivated in the sterile modified Czapek Dox liquid medium of the following composition (g L^{-1}): $NaNO_3$ – 3, K_2HPO_4 – 1, $MgSO_4 \times 7H_2O$ – 0.25, $FeSO_4 \times 7H_2O$ – 0.01, sucrose – 30, distilled water up to 1000 mL (control-K) and the same medium with additional 5 g of detergent to obtain a concentration of 0.5% (medium D5).

Erlenmeyer flasks with liquid growth medium were sterilized at 121°C for 20 min (autoclave pressure, 0.14 MPa). The pH was adjusted before sterilization at about 4.70 with 1 mol dm^{-3} HCl.

Inoculation and sampling

The liquid growth media were stored in Erlenmeyer flasks (200 mL of medium in 250 mL flask). One positive control without detergent with spores, one test flask with detergent and with spores and one negative control with detergent but without spores were used in this experiment. Inoculation of media occurred with 2 mL of spore suspension (5×106 conidia mL^{-1}). Erlenmeyer flasks in three replicates were placed on an electric shaker (Kinetor, Ljubljana, Slovenia) thus enabling uniform and constant mixing.

All experiments were carried out at room temperature, under alternate light and dark for 16 days. Sampling was started three days after inoculation and repeated every third day until the end of the experiment. Mycelium as removed by filtration through Whatman filter paper No.1 and mycelia dry weight was determined. Filtrate was harvested by centrifugation at 10,000g for 10 min (4°C) and the supernatant was used as crude enzyme extract.

Measurement of pH and redox potential

pH and redox potential were measured by digital electric pH meter (PHS-3BW Microprocessor pH/mV/temperature meter) type of Bante with a glass electrode model 65-1.

Determination of dry weight biomass

The mycelia previously removed from the fermentation broth were washed with sterile distilled deionized water several times. Both filter paper and mycelia were then dried in an oven at 80°C to a constant weight of the dry of mycelia and filter paper.

The experiments were performed using monosporial culture of the fungi *Aspergillus niger* van Tiegheme isolated from the river Lepenica (Serbia) on a wastewater outpouring site. Identification of the culture was done at the Faculty

of Biology, Belgrade, Laboratory for algae, fungi and lichens. Monosporial culture of the fungi was obtained by the method of exhaustion on poor potato-dextrose agar [24].

The method can be summarized as follows: fungi were inoculated into a flask that contained a chemically-defined microbial growth medium and the surfactant to be tested. The fungi were grown in the sterile liquid nutrient medium according to Czapek consistING of: 3g $NaNO_3$, 1g K_2HPO_4, 1g $MgSO_4$, 0.25g $MgSO_4 \times 7H_2O$, 0.01g $FeSO_4 \times 7H_2O$ and 30g saccharose, dissolved in 1000 ml of distilled water. Detergent designated D, ethoxyl-oleylcetyl alcohol (AOC) and sodium tripolyphosphate (TPP) were added (1%) and the flasks were incubated for 4-8 days. The flasks containing 200ml of medium were uniformly and constantly shaken on a Kinetor shaker at room temperature under conditions of alternate light-dark cycles [25]. The sterility of the nutrient medium was tested using mesopeptone agar.

For the determination of free organic acids 10ml of medium was taken and mixed with 50ml of ethanol. After incubation at 70°C in a water bath for 1-1.5 h, the mixture was filtered through a special filter. The filtrate was concentrated at 50°C - 60°C under reduced pressure to 40ml, transferred to a volumetric flask and made up to 100ml after addition of a teaspoon of active charcoal. After standing in a water bath for 30-45 min at 70°C, 10ml aliquots of filtrate were taken for the determination of the free organic acids by titration with 0.1 M NaOH in the presence of phenolphthalein as indicator [26-28].

Isolation and identification of Trichoderma harzianum Rifai

The selected fungus species originated from wastewater samples of the Rasina River, downstream where the industrial wastewaters of the factory Henkel (Kruševac, Serbia) discharge into river. Sample of wastewater was taken in late May 2010.

The identification of the fungus *T. harzianum Rifai* was based primarily on the macroscopic and microscopic morphology and was carried out by systematic key. The fungus was maintained on potato-dextrose-agar (PDA) slant grown at 30°C, stored at 4±0.5°C, and subcultured monthly in sterile conditions.

Fermentation conditions

During the experiment, the fungus was cultivated in a sterile modified Czapek Dox liquid medium of the following composition (g L^{-1}): $NaNO_3$-3, K_2HPO_4-1, $MgSO_4 \times 7H_2O$-0.25, $FeSO_4 \times 7H_2O$-0.01, sucrose-30, distilled water up to1000mL (control-C) and the same medium with additional 3g of detergent to obtain a concentration of 0.3% (medium D3). Erlenmeyer flasks with liquid growth medium (200mL of medium in 250mL flask) were sterilized at 121°C for 20 min (autoclave pressure, 0.14 MPa). The pH was adjusted before sterilization at about 4.70 with 1 mol L^{-1} HCl. After addition of detergent to the liquid growth medium the pH value of the medium was measured again.

Inoculation and sampling

One positive control without detergent with spores, one test flask with detergent and with spores and one negative control with detergent but without spores were used in this experiment. Inoculation of media occurred with 2mL spore suspension (5×106 conidia mL^{-1}). Erlenmeyer flasks in three replicates were placed on an electric shaker thus enabling uniform and constant mixing. All experiments were carried out at room temperature, under alternate light and dark for 16 days. Sampling was started three days after inoculation and repeated every third day until the end of the experiment. Mycelium was removed by filtration through Whatman filter paper No.1. Filtrate was harvested by centrifugation at 10000g for 10 min (4°C) and the supernatant was used as crude enzyme extract.

Measurement of pH and redox potential

pH and redox potential were measured on a digital electric pH meter (PHS-3BW Microprocessor pH/mV/temperature meter) type Bante with a glass electrode model 65-1.

Determination of biomass dry weight

The biomass dry weight of the mycelia was determined according to the procedure described in Jakovljevic *et al*. (2014) [9].

Statistical analysis

All experiments were performed in triplicate and results were expressed as means ± standard deviation. For statistical analysis, the following tests were used: Mann-Whitney, Kruskal-Wallis and test for correlation coefficient by SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. XIII, 2004). Coefficient of correlation was determined at the levels of significance 0.05 and 0.01.

RESULTS AND DISCUSSIONS

The influence of the detergent Merix (Merima, Kruševac, Serbia) and its components (ethoxyl-oleyl-cetyl alcohol and sodium tripolyphosphate) on the metabolic activity of the fungi *Mucor plumbeus*, *Aspergillus niger* and *Trichoderma harzianum* was studied. The metabolic changes of bioproduction of different organic compounds in various aging steps of the fungi in the presence of pollutant was investigated. These fungi were isolated from the river Lepenica (Serbia) on the wastewater outpouring site and were chosen because they were the most abundant there. The fungi were then grown in a liquid nutrient medium according to Czapek, where 1% of detergent or its component was added, during the incubation period of 4-8 days after inoculation.

Metabolic activity of *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum* grown in such liquid nutrient medium and control medium was monitored over the following biochemical parameters: amounts of free and total organic acids, pH values, redox potential and biomass.

Organic acids play a key role in alkali tolerance, especially for intracellular ionic homeostasis. Organic acids produced by fungi into the medium can exist in free (FOA) and in bound form. The sum of the amounts of both free and bound organic acids represents the amount of total organic acids (TOA). Figures 1 and 2 show the concentrations of free organic acids (FOA) and of total organic acids (TOA) in the fermentation broth measured during the fungal growth. Production of free and total organic acids increased in all cases (for all three fungi) at the end of the experimental period (Table 1, Figure 1).

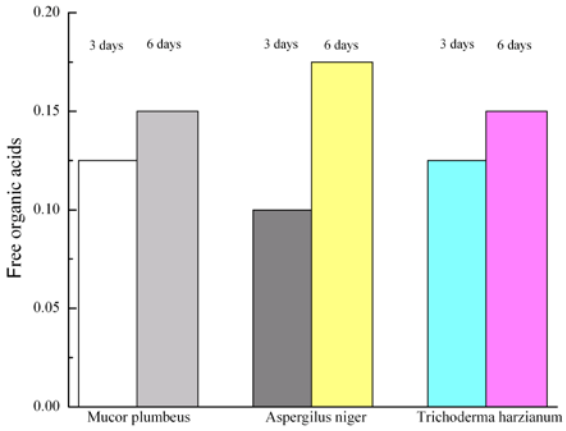

Fig. 1. Change in the concentration of free organic acids (expressed as %) in a nutrient medium with detergent.

After three days of observation, the lowest concentration of free acid was measured for the fungus *Aspergillus niger*, and its value is 0.100%. For the same period, *Mucor plumbeus* and *Trichoderma harzianum*, showed a higher content of free acid, 0.125%. On the sixth day of observation, for *Mucor plumbeus* and *Trichoderma harzianum*, the content of free organic acids was 0.150%, and the highest concentration of free acid was displayed b+y *Aspergillus niger*. Based on the measured values of the concentration of free organic acids, we can see that *Aspergillus niger*, after three days had the lowest value and after six days the highest value.

Similar values for the examined fungi have been found by other authors. The concentration of free organic acids in *Aspergillus niger* at an early growth stage (day 3) was lower than 0.100% and at the sixth day - less than 0.150%. The lowest value was recorded during the autolysis (day 9) and amounted to 0.380%, the highest being at the 16th day - 1.200%. For *Trihoderma harzianum* the concentration of free acids at the third day was the lowest (0.03%) and at the 16th day – the highest, 0.12%. In the same research the concentration of free organic acids in *Mucor racemosus* was determined which was the highest - 0.14%, recorded during the exponential growth phase [30].

In other studies, for *Aspergillus niger* higher concentrations of free organic acids in the substrate with detergent were recorded and their value after 4 and 7 days amounted to 1.80% and 2.50%, respectively. In the same research, the concentration of free organic acids in *Trichoderma viride* was determined after 4 days - 1.00%, and after 7 days - 1.20%, which were higher than those recorded for *Trichoderma harzianum* [27].

In Table 2 and on Figure 2 the total free acids for all 3 fungi *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum* are shown.

In studies by other authors slightly lower values of the content of total organic acids have been noticed. The concentration of total organic acids for *Aspergillus niger* after the third day was below 0.6%, while after the sixth day the concentration was about 0.70%. For *Trihoderma harzianum* the highest value recorded during exponential growth was 0.69% to 1.06% after the sixth day. For *Mucor racemosus* the largest concentration was observed at the sixth day - 13.01% [30]. In a similar study, the total content of organic acids in the substrate with a detergent, for *Aspergillus niger* after the 4th day amounted to 2.60%, while at the 7th day it was slightly lower, 2.30%. For *Trichoderma viride*, the total content of organic acids in the medium with

detergent after the 4th day was 1.50% and after the 7th day - 2.00% [27].

Chemical compositions of growth media and experimental conditions have influence on fungal development and total biomass. Many investigations showed that the Czapek Dox liquid medium has good properties for fungal cultivation and high biomass production [14].

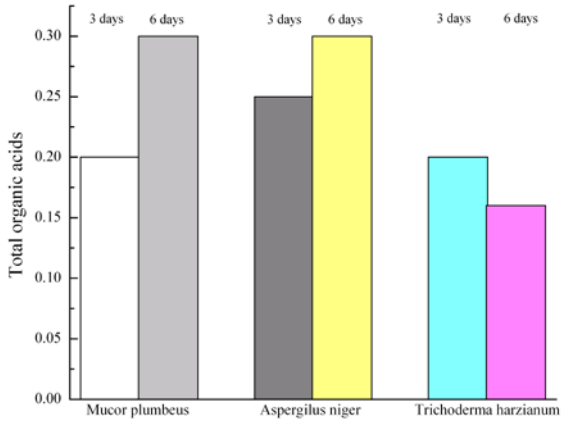

Fig.2. Total organic acids for *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum*

The fungus cultivated in a medium without detergent (K medium) showed similar biomass amount. But after the influence of detergent (D), the biomass amount rapidly decreased (Table 3, Figure 3).

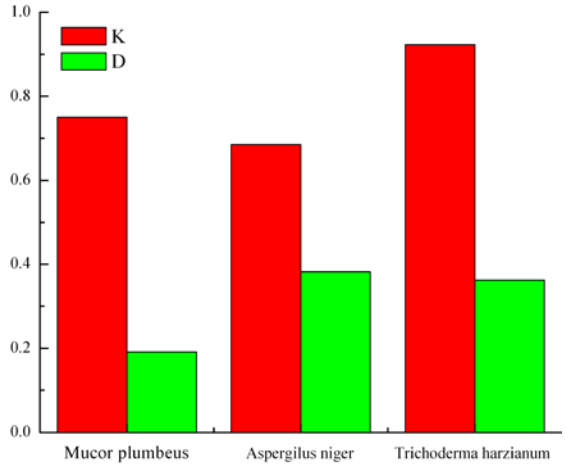

Fig.3. Biomass (g) *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum*

In this study the changes in pH and redox potential during fungal growth were estimated because these parameters are very important for regular growth and development and affect the morph-physiological characteristics and biochemical properties of the microorganisms.

The optimum external pH for fungal growth was under acidic conditions from 4.5 to 5. Fungi generally alter the pH of the medium in which they grow, due to uptake of anions or cations from the medium [25,26]. Therefore, the various changes witnessed in the pH values of the culture media are a result of the utilization of nutrients from the growth media [27]. Based on literature data, the fungi do not develop at pH above 9. This study provides evidence that *M. Racemosus Fresenius* could tolerate a wide range of environmental pH, from 4.75 to 9.80. Figure 4 shows that the pH values of the fermentation broth changed during the fungus growth from inoculation until the 16th day. The initial pH values of the media were 4.75 in K medium and 9.80 in D5 medium before inoculation. Also, one negative control (nkD5) with detergent but without spores was tested. The pH values of inoculated growth media changed in relation to their composition and growth phases of fungus. pH value of K medium increasing from inoculation until the 6th day. During stationary and autolysis phases, the pH value slightly decreased but these changes were not statistically significant. In contrast, the pH value of the D5 medium decreased in the exponential growth phase. The largest decrease in the pH value was observed in the medium D5 between the 3rd and 6th day (from 9.36 to 6.46 units) which corresponds to primary exponential growth phase. These changes of pH were less expressed in the secondary exponential growth phase. Interestingly, the final pH values of the different media were very similar although the differences between the initial pH values were very significant.

In the papers of other authors it was observed that the pH value of the substrate K for *Aspergillus niger* gradually decreased during the growth and development of the fungus, with the largest reduction recorded in the period up to the 9th day of inoculation, ranging from pH 4.80 to pH 2.53, while the least pH change was observed from the 9th to the 16th day. In the medium D3, the biggest decrease in pH was in the primary exponential phase from the 3rd to the 6th day, with pH in the range from 9.13 to pH 6.49. For *Trichoderma harzianum* in the substrate K the largest pH increase was during the first three days from 4.80 to 5.40 and the largest reduction from the 3rd to the 6th day from 5.40 to 4.82 while the pH changes during the stationary phase and autolysis were much lower. In the medium D3, the pH value displayed the least changes in the initial stage of development fungi from inoculation to the 6th day. In the phase of exponential growth, from 6th to 9th day, there was the largest change in pH from 6.07 to 9.05, and the stationary phase pH changes were minimal. For *Mucor racemosus* the pH value of the control substrate K gradually increased to the 6th day, while

during the stationary phase and autolysis it decreased. The largest decrease in pH in the substrate D3 was during the inoculation period to the 3rd day with pH changing from 9.35 to 6.24, and in the substrate D5 it was from the 3rd to the 6th day with pH changing from 9.36 to 6.46. In the secondary exponential growth phase, the pH changes were significantly lower [30].

Table 1. Changes in concentration of free organic acids (expressed as %) in the nutrient medium with detergent.

Fungus in nutrient medium with detergent	*Mucor plumbeus*		*Aspergillus niger*		*Trichoderma harzianum*	
Incubation period	day 3	day 6	day 3	day 6	day 3	day 6
Concentration of free organic acids (%)	0.125	0.150	0.100	0.175	0.125	0.150

Table 2. Total organic acids for *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum.*

Fungus in nutrient medium with detergent	*Mucor plumbeus*		*Aspergillus niger*		*Trichoderma harzianum*	
Incubation period	day 3	day 6	day 3	day 6	day 3	day 6
Concentration of free organic acids (%)	0.200	0.300	0.250	0.300	0.200	0.160

Table 3. Biomass (g) of *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum*

	Mucor plumbeus		*Aspergillus niger*		*Trichoderma harzianum*	
Incubation period	Biomass content in the control medium (g)	Biomass content medium with detergent (g)	Biomass content in the control medium (g)	Biomass content medium with detergent (g)	Biomass content in the control medium (g)	Biomass content medium with detergent (g)
16th day	0.7501	0.1915	0.6852	0.3823	0.9226	0.3627

Table 4. pH values of *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum*

	Mucor plumbeus		*Aspergillus niger*		*Trichoderma harzianum*	
Incubation period	pH value in the control medium	pH value in medium with detergent	pH value in the control medium	pH value in medium with detergent	pH value in the control medium	pH value in medium with detergent
3rd day	4.76	9.19	6.13	9.10	3.71	9.00
6th day	3.95	6.20	6.70	6.93	3.58	8.92
9th day	3.41	6.33	6.89	6.22	2.62	5.86
12th day	3.43	6.15	7.17	6.20	2.42	5.97
16th day	3.19	5.88	7.39	6.25	2.36	5.19

Table 5. Redox potential of *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum.*

	Mucor plumbeus		*Aspergillus niger*		*Trichoderma harzianum*	
Incubation period	Redox potential in the control medium (mV)	Redox potential in medium with detergent (mV)	Redox potential in the control medium (mV)	Redox potential in medium with detergent (mV)	Redox potential in the control medium (mV)	Redox potential in medium with detergent (mV)
3rd day	131	123	51	118	191	112
6th day	176	44	18	4	197	106
9th day	194	37	4	43	236	63
12th day	193	56	11	53	243	66
16th day	200	66	23	46	244	104

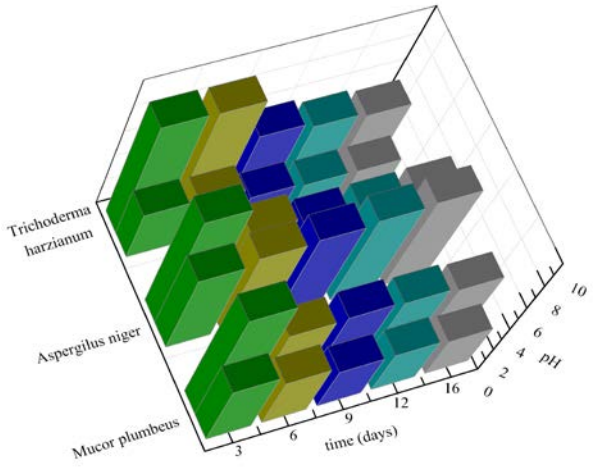

Fig. 4. pH values of *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum*

Table 5 and Figure 5 illustrate that the redox potential values of fermentation broth changed during the growth of fungus from inoculation until the 16th day. The initial redox potential values were 130 mV in K and–148 mV in D5 medium before inoculation. One negative control (nkD5) with detergent but without spores was also tested. The redox potential of the K medium decreased during the exponential growth phase (from inoculation until the 6th day), whereas it slightly increased throughout stationary and autolysis phases. During the biphasic exponential growth of the fungus in D5 medium, the redox potential increased more intensively in the primary than in the secondary exponential growth phase. The decrease in redox potential was measured in D5 medium on the 9th day only.

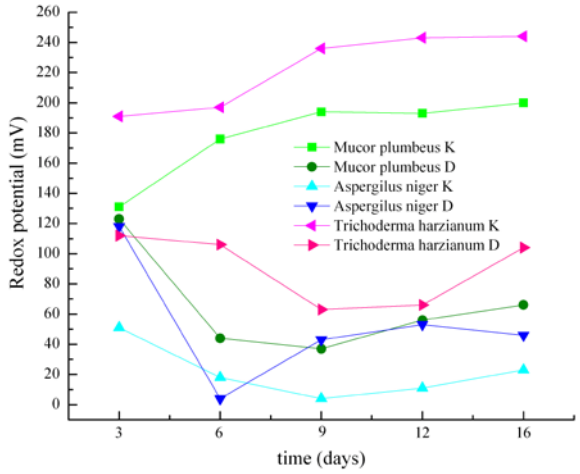

Fig. 5. Redox potential of *Mucor plumbeus, Aspergillus niger* and *Trichoderma harzianum.*

In other studies, it was observed for *Aspergillus niger* that the value of the redox potential gradually decreased from the inoculation period until the end of the experiment, except for the 3rd and 6th day. The values of the redox potential of the medium D3 have increased from the moment of inoculation to the 9th day, while the largest change in redox potential was noticed in the phase of exponential growth. For *Trichoderma harzianum*, the value of the redox potential of the control surfaces, decreased from the beginning inoculation till the end of the experiment with the exception of the 3rd and 6th day. In the medium D3, the redox potential values gradually increased during the period of cultivation and the largest changes were noticed between the 6th and 9th day. The value of the redox potential during the growth of the fungus *Mucor racemosus* was measured in the control substrates and in the media with a detergent concentration of 0.3% and 0.5%. The value of the redox potential in the control medium rapidly decreased from inoculation to the 6th day, with a slight increase during the stationary phase and autolysis. The redox potential in the substrate D3 gradually increased until the end of the experiment and the largest changes were noticed in the exponential growth phase (day 3), in addition to the 6th and 9th day. The redox potential of in the substrate D5 also gradually increased until the end of the experiment, with the exception of the 6th and 9th day, and the largest changes were from the 3rd to the 6th day and from the 9th to the 16th day [30].

In this study it was observed that for *Aspergillus niger* the redox potential in the control substrate also gradually decreased from the beginning of inoculation to the 9th day when the lowest value was recorded, then there was a gradual increase in the value of the redox potential from the 12th to the 16th day of the experiment. As opposed to the previous studies, the value of the redox potential of the medium D3 decreased with the largest decrease was recorded at the 3rd day and 6th day. For *Trihoderma harzianum*, the redox potential value in the control medium gradually increased from inoculation until the end of the experiment, in contrast to the above studies, where the value of the redox potential gradually decreased. In the medium D3, the redox potential value gradually decreased during the experiment, except from the 12th to the 16th day. For *Mucor racemosus*, the value of the redox potential in the control medium gradually decreased until the end of the experiment, while that in the substrate D3 gradually decreased with the exception the 12th and the16th day when there was a gradual increase.

CONCLUSION

The content of free organic acids for *Aspergillus niger* amounted to 0.100% on the third day and 0.175% on the sixth day. For the fungus *Mucor plumbeus* the content of free organic acids on the third day was 0.125%, while on the sixth day - 0.150%. The content of free organic acids for *Trichoderma harzianum* was 0.125% after the third day and 0.150% after the sixth day.

In the medium with the detergent the content of total organic acids in the examined fungi was determined on the third and the sixth day after the beginning of inoculation. The content of total organic acids for *Aspergillus niger* after the third day was 0.250%, and after the sixth day - 0.300%. The content of total organic acids for *Mucor plumbeus* at the third day amounted to 0.200% and on the sixth day - 0.300%. For the fungus *Trichoderma harzianum,* at the third day the content of total organic acids was 0.200%, while at the sixth day - 0.160%.

The fungi cultivated in a medium without detergent showed similar biomass. But after the influence of detergent (D), the biomass rapidly decreased. At the end of the experiment, for *Mucor plumbeus*, which grew in a medium without detergent, the biomass amounted to 0.7501g, the biomass content in the substrate with a detergent was 0.1915g. For *Aspergillus niger*, the control medium at the end of the experiment had a biomass content of 0.6852g and in the medium with detergent - 0.3823g. In the same conditions, *Trihoderma harzianum* had 0.9226g of biomass in the control medium, while in the medium with detergent - 0.3627g.

The pH value of *Mucor plumbeus* in a medium without detergent gradually decreased from the beginning of inoculation to the end of the experiment. The pH value of the medium with a detergent, was the largest on the third day - 9.19 and then decreased to pH 5.88. *Aspergillus niger* in the control medium had a minimum value of pH on the third day - 6.13, then pH increased to a value of 7.39 at the end of the 16th day. The pH value in a medium with a detergent decreased from 9.10 to 6.25. The pH value of *Trichoderma harzianum* in the control medium decreased from 3.71 to p 2.36, and in the medium containing detergent from 9.00 to 5.19.

The redox potential for *Mucor plumbeus* in the control medium decreased from 131 mV at the beginning of the inoculation to 200 mV at the end of the experiment. In a medium with detergent, for *Aspergillus niger* the redox potential at the third day was the largest one - 118 mV, then it rapidly decreased till the 6th day and increased again to 53 mV at the 12th day. The redox potential in the control medium for *Aspergillus niger* was the largest at the third day (51 mV), then it first decreased to 4 mV and then increased to 23mV. The redox potential of the control medium for *Trichoderma harzianum* was the lowest on the third day - 191mV and gradually increased to 244mV on the 16th day. The redox potential of *Trichoderma harzianum* in the medium with detergent was the largest at the third day - 112 mV, then it decreased to 66 mV (day 12), and increased to 104mV on the 16th day.

The presence of a detergent in the nutrient medium and its degradation products during the fermentation of the fungus, influenced the biochemical changes of all examined parameters. Detergent influenced the inhibition of fungal biomass at a different percentage, depending on the type of fungi. All fungi degraded a detergent concentration of 0.3%, except the *M. racemosus* degrading a detergent concentration of 0.5%. Research has indicated that the tested fungi can decompose the detergent and its products, indicating the possibility of their use for that purpose.

REFERENCES

1. J.L. Sanz, E. Culubret, J. De Ferrer, A. Moreno, J.L. Berna, *Biodegradation*, **14**, 57 (2003).
2. P. Bonin, C. Cravo-Laureau, V. Michotey, A. Hirchler-Rea, *Ophelia*, **58**, 243 (2004).
3. http://www.scielo.cl /scielo.php?pid=S0717-345819.
4. G. Saucedo-Castañeda, B.K. Lonsane, J.M. Navarro, S. Roussos, M. Raimbault, *Applied Biochemistry and Biotechnology*, **36**, 47 (1992).
5. M. Raimbault, Fermentation en milieu solide: croissance de champignons filamenteux sur substrats amylacés. Série Travaux et Documents, ORSTOM, Paris, 1981.
6. G. Saucedo-Castañeda, B.K. Lonsane, J.M. Navarro, S. Roussos, M. Raimbault, *Process Biochemistry*, **27**, 97 (1992).
7. V.G. Lilly, H.L. Barnett, Physiology of the fungi, McGraw--Hill Book Co., New York, 1951, p. 464.
8. P. Borgia, P.S. Sypherd, *J. Bacteriol.*, **130**, 812 (1977).
9. P.A. Micheli, Nova Plantarum Genera, Florentiae, 1729.
10. O.P. Ward, W.M. Qin, J. Dhanjoon, J. Ye, A. Singh, *Advances in Applied Microbiology*, **58**, 1 (2005).
11. J.L. Brookman, D.W. Denning, *Current Opinion in Biotechnology*, **3**, 468 (2000).
12. R.P. de Vries, *Applied Microbiology and Biotechnology*, **61**, 10 (2003).
13. O.B. Fawole, S.A. Odunfa, *International Biodeterioration & Biodegradation*, **52**, 223 (2003).

14. L. Wang, D. Ridgway, T. Gu, M. Moo-Young, *Biotechnology Advances*, **23**, 115 (2005).
15. N.C. Mhetras, K. Bastawde, D.V. Gokhale, *Bioresource Technology*, **100**, 1486 (2009).
16. B. Joseph, P.W. Ramteke, G. Thomas, *Biotechnology Advances*, **26**, 457 (2008).
17. F. Hasan, A.A. Shah, A. Hameed, *Enzyme and Microbial Technology*, **39**, 235 (2006).
18. M.J. Tailor, T. Richardson, *Advances in Applied Microbiology*, **25**, 7(1979).
19. F.J. Contesini, D.B. Lopes, G.A. Macedo, M. da Graca Nascimento, P. de Oliveira Carvalho, *Journal of Molecular Catalysis B: Enzymatic*, **67**, 163 (2010).
20. S. Mitidieri, A.H.S. Martinelli, A. Schrank, M.H. Vainstein, *Bioresource Technology*, **97**, 1217 (2006).
21. R. Hermosa, A. Viterbo, I. Chet, E. Monte, *Microbiology*, **158,** 17 (2012).
22. M.S.Y. Haddadin, J. Haddadin, O.I. Arabiyat, B. Hattar, *Bioresour. Technol.*, **100,** 4773 (2009).
23. A. Katayama, F. Matsumura, *Environ. Toxicol. Chem.*, **12**, 1059 (1993).
24. M.V. Gorlenko, D.V. Sokolov, Plant growing. **II**, Prosvetenije, Moscow, 1976.
25. J. Stojanović, Influence of detergent on biochemical properties of some fungi *in vitro*. Ph.D. Dissertation. Faculty of Science, Kragujevac, 1990.
26. D. Veličković, Contribution to study of aminoacidic composition dynamics of protein complex and aminoacids in apple fruits during the vegetative period and storage. Ph.D. Dissertation. University of Belgrade, Belgrade,1971.
27. J. Stojanović, M. Stojanović, A. Milovanović, *Acta Veterinaria Beograd*, **51**, 171 (2001).
28. J. Stojanović, D. Veličković, J. Vučetić, *Acta Veterinaria Beograd*, **52**, 267 (2002).
29. V.D. Jakovljević, J.M. Milićević J.D. Stojanović M.M. Vrvić, *Chem. Ind. Chem. Eng. Q.*, **20**, 587 (2014).
30. V.D. Jakovljevic, Biohemical characteristics of selected type of fungi in function biodegradation detergent. Ph.D. Dissertation. University of Kragujevac, Kragujevac, 2014.
31. J. Mehta, M. Jakhetia, S. Choudhary, J. Mirza, D. Sharma, P. Khatri, *Eur. J. Exp. Biol.*, **2**, 2061 (2012).
32. E. Moore-Landecker, Fundamentals of Fungi, Prentice Hall, Upper Saddle River, New York, 1996, p.574.
33. D.H. Griffin, Fungi Physiology, Wiley-Liss, New York, 1994, p.468.
34. M. Orlowski, *Microbiol. Rev*., **55**, 234 (1991).

СРАВНИТЕЛНО ИЗСЛЕДВАНЕ НА НЯКОИ БИОХИМИЧНИ ПАРАМЕТРИ НА ГЪБИ *Mucor plumbeus, Aspergillus niger* И *Trichoderma harzianum*

И. Матович-Пурич[1], Д. Печарски[2], З. Югович[3], Д. Йовичич[4], Д. Джорджевич[5], П. Маскович[6]

[1]Медицинско училище, Епископ Никифор Максимович,№. 8, Чачак, Сърбия
[2]Висше училище за професионално здравеопазване, Кара Душана 254, Белград, Сърбия
[3]Висше санитарно здравно училище за професионални изследвания "Визан", Тошин бунар 7а, Белград, Сърбия
[4]Университет Сингидунум, Факултет за приложна екология - Футура, Пожешка 83, Белград, Сърбия
[5]Университет Ниш, Факултет за науки и математиката, Вишеградска 33, Ниш, Сърбия
[6]Университет Крагуевац, Факултет по агрономия, Чачак, Сърбия

Получена на 12 септември 2016 г .; приета на 21 октомври 2016 г.

(Резюме)

Целта на това изследване е да се изследва ефектът от високите концентрации на търговския детергент "Merix" (Хенкел, Крушевац) върху растежа, развитието и биохимичните характеристики на тестваните гъби, изолирани от канализационни и промишлени отпадъчни води. Метаболитната активност на *Mucor plumbeus, Aspergillus niger* и *Trichoderma harzianum*, отглеждани в такава течна хранителна среда и контролна среда се следи по следните биохимични параметри: количества свободни и общи органични киселини, pH стойности, редукционен потенциал и биомаса. В зависимост от видовете гъбички има инхибиране на растежа на биомасата при различни концентрации на детергента в периода от третия до шестия ден. Концентрацията на детергента от 0,3% се разлага във всички тествани смеси гъби - детергенти.