CI&CEQ
Chemical Industry & Chemical Engineering Quarterly

Available on line at
Association of the Chemical Engineers of Serbia *AChE*
www.ache.org.rs/CICEQ

Chem. Ind. Chem. Eng. Q. 20 (4) 587–595 (2014) CI&CEQ

VIOLETA D JAKOVLJEVIĆ[1]
JASMINA M MILIĆEVIĆ[1]
JELICA D STOJANOVIĆ[1]
MIROSLAV M VRVIĆ[2]

[1]Institute for Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia
[2]Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

SCIENTIFIC PAPER

UDC 661.185.6(497.11):582.281:66:544

DOI 10.2298/CICEQ130922002J

THE ABILITY OF FUNGUS *Mucor racemosus* FRESENIUS TO DEGRADE HIGH CONCENTRATION OF DETERGENT

Article Highlights

- Native isolate of fungus *Mucor racemosus* Fresenius was tested to high concentration of detergent
- Influence of detergent on changes of fungal biochemical parameters
- Fungus decomposed 62% of anionic surfactant during 16 days, which confirmed by MBAS assay
- Detergent at concentration of 0.5% reduced about 60% of fungal alkaline phosphatase activity

Abstract

The ability of fungus Mucor racemosus Fresenius to decompose high concentration of commercial detergent (Merix, Henkel, Serbia) was investigated in this study. Fungus was cultivated in liquid growth medium by Czapek with addition of detergent at concentration 0.5% during 16 days. The biochemical changes of pH, redox potential, amount of free and total organic acids, and activity of alkaline phosphatase were evaluated by analysis of fermentation broth. Simultaneously, biodegradation percentage of anionic surfactant of tested detergent was confirmed by MBAS assay. At the same time, the influence of detergent on fungal growth and total dry weight biomass was determined. Detergent addition at concentration 0.5% resulted in a decrease in pH value and increase in redox potential as well as increase of free and total organic acids. Enzyme activity of alkaline phosphatase was reduced by detergent at concentration 0.5%. The fungus was decomposed about 62% of anionic surfactant during 16 day. Due to the fungus, higher dry weight biomass (53%) was produced compared to the control.

Keywords: biomass, biodegradation of detergent, enzyme activity, organic acids, pH, redox potential.

For over 2000 years, mankind has used surface-active components or their ingredients in the various aspects of daily life, personal care products, laundry washing, chemical cleaning and cosmetics. Most of the surfactants are used in the form of detergent powders. A modern detergent powder is a complicated multicomponent mixture consisting of surfactants, builders, bleach, enzymes and auxiliaries [1,2]. Usually, a detergent powder contains about 20--25% surfactants.

Correspondence: V. Jakovljević, Institute for Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34 000 Kragujevac, Serbia.
E-mail: jakovljevicvioleta@gmail.com
Paper received: 22 September, 2013
Paper revised: 5 December, 2013
Paper accepted:13 December, 2013

According to statistics, the annual world production of surfactants is about 12.5 million tons, and in the EU it is about 2.99 million tons. Approximately 64% of surfactant is produced in detergents for washing and cleaning. It is estimated that the rate of surfactant production increases annually by about 0.5 million tons. Increased usage of detergents in households has led to their accumulation in wastewater and natural aquatic ecosystems, causing many harmful effects on microbial communities and hydro-bionics [3]. The main condition for the relatively safe usage of surfactants is their easy and ultimate biodegradation [4]. Surfactants used in detergent formulation should be degraded at least 80% in terms of primary biodegradation. Detergents as pollutants get into the environment through industrial and municipal wastewater, pesticide application or deposit of waste activated

sludge [5]. Discharging of sewage and surfactants in their final appearance in the sediment, the toxicity to aquatic organisms and bioaccumulation in the food chain, along with emissions of CO_2, SO_2 and NO_x, are the main problem today. Products with high contents of surfactants, such as detergents, inhibit the growth of unicellular algae, impede the process of purifying water creating foam, which makes it difficult to dissolve oxygen that is necessary for living things breathing and photosynthesis, and cause eutrophication [6]. Due to these facts, the maximal allowable concentration of detergent in wastewater discharged into the public sewage is 4 and 0.5 mg L^{-1} in natural recipients, according to Legislative acts [7]. Numerous studies have focused on the discovery of new biosurfactants that would replace the synthetic ones as well as on the development of new species of microorganisms that may degrade synthetic surfactants and complex chemical compounds in nature. Filamentous fungi are attractive microorganisms for the study of biodegradation of organic matter due to their well-developed defense mechanisms and the structure of the cell wall. Fungi are recognizing for their superior capability to produce a large variety of extracellular proteins, organic acids and the other metabolites, as a result of adaptation to severe environmental constraints [8]. Many reports have shown that surfactants change conformation of protein, which can influence the enzymatic activity, stability and specificity or disrupt the structure of cell membranes, depending on the concentration of surfactants and type of microorganism.

Mucor racemosus Fresenius is a dimorphic fungus (genus *Mucor*) whose growth 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 [9]. 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 objective of this study was to evaluate the ability of fungus *M. racemosus* Fresenius to degrade commercial powder detergent (Merix, Henkel, Kruševac, Serbia) at very high concentration (5 g L^{-1}), which is thousands fold higher than the regulated concentrations that may be found in sewage water and natural recipients and lethal for the most microorganisms. Simultaneously, we investigated the influence of detergent on the growth and biochemical characteristics of fungus.

EXPERIMENTAL

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 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 is disposed of in a refrigerator at 4 °C. Within 24 h, the different dilutions of 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 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 subculture 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, $MgSO_4 \cdot 7H_2O$ – 0.25, $FeSO_4 \cdot 7H_2O$ – 0.01, sucrose – 30, distilled water up to 1000 mL (control-K) and the same medium with additional 5 g of detergent to obtain 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 control was adjusted before sterilization 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 spore suspension (5×10^6 conidia mL^{-1}). Erlenmeyer flasks in three replicates were placed on an electric shaker (Kinetor-m, 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 was removed by filtration through Whatman filter paper No. 1 and mycelial dry weight was determined.

Filtrate was harvested by centrifugation at 10,000*g* 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/ /Temperaturemeter) type of Bante with glass electrode model 65-1.

Determination of dry weight biomass

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

Determination of anionic surfactant

Anionic surfactant was determined by spectrophotometric methods using methylene blue. The concentration of methylene blue-active substance (MBAS) in the detergents was determined according to Standard Methods for the Examination of water and wastewater [10]. Absorbance measurements of the extracts were done using Perkin-Elmer Lambda 25 UV-Vis spectrophotometer set at 652 nm wavelength against blank chloroform. The concentrations of the residual surfactant present in test detergent in terms of MBAS were calculated using calibration curve with the SDS as the standard. The percentage of degradation was then calculated using the Eq. (1):

$$\%\,\text{Degradation} = 100 - \\ -[(A_{625}\,\text{exp} - A_{625}\,\text{blank}) / A_{625}\,\text{std}] \times 100 \quad (1)$$

where A_{625}exp is absorbance of test sample, A_{625}blank is absorbance of blank sample and A_{625}std is absorbance of standard sample at 625 nm.

Determination of free and total organic acids concentrations

Concentrations of free and total organic acids were determined by ion exchange chromatography method. 50 mL of ethanol (70%) was added to 10 mL of fermentation broth and the reaction mix was incubated at 70 °C in water bath for 1 h. The mixture was filtered through Whatman filter paper No. 1 and filtrate was concentrated at 50–60 °C under reduced pressure to final extract volume of 40 mL. Active charcoal was added to extract following by incubation 30 min in the water bath at 70 °C. After incubation, the extract was filtrated to remove active charcoal; the residue was made up to a volume of 100 mL with distilled water. 10 mL aliquots of filtrate were sampling for determination of free organic acids concentration by titration 0.1 mol dm^{-3} NaOH. Phenolphthalein (0.1%) was used as indicator. The residual of sampling (90 mL) was passed through a cationic column (Amberlite IR-120) previously activated, to the volumetric flask of 250 mL. By washing the column with distilled water volumetric flask was supplemented to 250 mL. To determination of concentration the total organic acids, 25 mL aliquots were sampling and titration was carried out as previously described [11]. The results were presented as a percentage.

Assay of alkaline phosphatase activity (EC 3.1.3.1)

Alkaline phosphatase activity was assayed with *β*-glycerophosphate as substrate. The reaction mixture contained an equal volume of 0.05 mol dm^{-3} glycol buffer (pH 9) with activator (Mg^{2+}), substrate and fermentation broth. After incubation at 37 °C for 30 min, the reaction was stopped by adding 10% TCA, and the reaction mixture was stored at ice for 15 min. NH_4-molybdate solution was used for color development and determination of liberated inorganic phosphate (*Pi*). The absorbance was measured using a Perkin-Elmer Lambda 25 UV-Vis spectrophotometer at 660 nm [13]. One unit of enzyme activity (IU) represented the amount of enzyme that released 1 μg of inorganic phosphate per min under the assay conditions.

Statistical analysis

All experiments were performed in triplicate and results were expressed as means ± standard deviation. For statistical analysis, were used the following tests: 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 tested at the level of significance 0.05 and 0.01.

RESULTS AND DISCUSSION

The influence of detergent at 0.5% concentration on the changes of pH, redox potential, the concentration of organic acids, enzyme activity and total dry weight biomass were examined. At the same time, fungal ability to degrade anionic surfactants of tested commercial detergent and utilize degradation products as carbon and energy sources was tested. These parameters were monitored during the different physiological growth phases of fungus *M. racemosus* Fresenius. The results are shown in Figures 1-6.

Chemical compositions of growth media and experimental conditions have influence on fungal

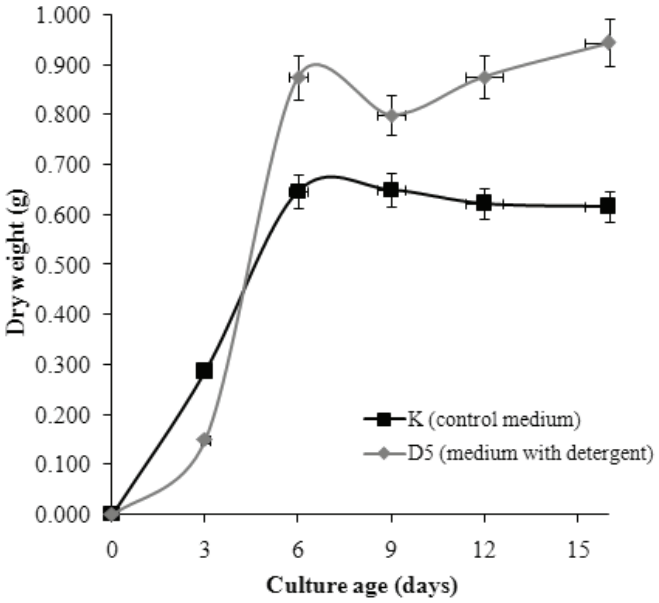

Figure 1. Display of growth and total dry weight biomass of fungus Mucor racemosus Fresenius cultivated in K (control medium) and D5 (medium with 0.5% detergent) during experimental period.

development and total biomass. Many investigations showed that the Czapek Dox liquid medium has good properties for fungal cultivation and high biomass production [14]. Chemical composition of growth media influenced the growth of *M. racemosus* Fresenius, as seen in Figure 1. The fungus cultivated in medium without detergent (K medium) showed monophasic exponential growth from inoculation until the stationary phase, which was achieved on the 9th day. After short stationary phase, autolysis began on 12th day and it influenced on slightly decreasing of biomass until the end of experiment. But changes of biomass weren't statistically significant in this period. An early fungal growth phase from inoculation until the 3rd day before exponential growth (from 3rd to 6th day) was observed in medium with 0.5% detergent. After the 6th day, growth was temporary inhibited and a second exponential growth phase was observed from the 9th to the 16th day. Detergent at 0.5% concentration stimulated growth and mycelial dry weight biomass in relation to the control.

Figure 2 shows the percentage of biodegradation of anionic component of tested detergent by fungus *M. racemosus* Fresenius. The biodegradation

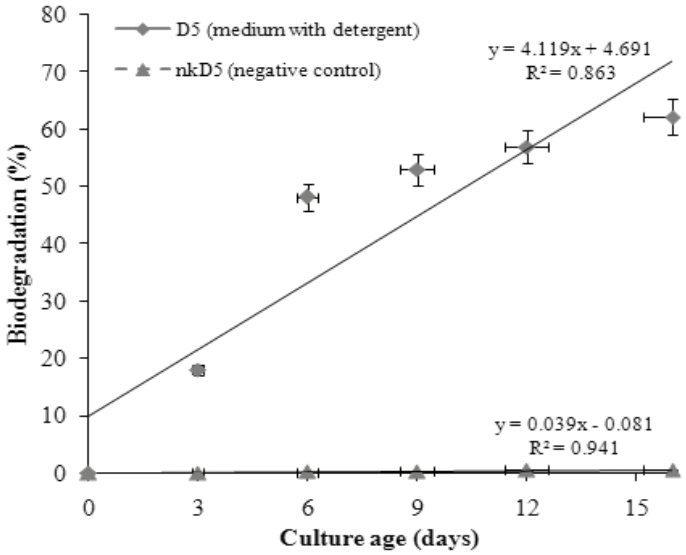

Figure 2. Percentage of biodegradation of anionic surfactants of detergent measured in fermentation broth of D5 (medium with 0.5% detergent) in compare to negative control (nkD5).

was evaluated in liquid medium supplemented with detergent at concentration of 0.5% (D5 medium) and with spores of fungus. Also, one negative control with detergent and without spores (nkD5) was evaluated. A negative control experiment was carried in order to exclude any reduction of anionic surfactant concentration caused by non-biological reactions such as adsorption of surfactants to the bottle wall. Negative control of detergent showed that anionic component of tested detergent had chemical stability and minimal adsorption to the bottles wall in experimental conditions. The tested detergent contained 20% of anionic surfactant, which was confirmed by MBAS assay. When concentration of anionic surfactant was expressed in μg mL^{-1} it was obtained that initial concentration of anionic surfactant was 1000 μg mL^{-1} in D5 medium. During first 3 days, the fungus decomposed 18% of initial concentration of anionic surfactant in D5 medium. The fungus decomposed the highest percentage of anionic surfactants (56.16%) during exponential growth phase from 3rd to 6th day. In the following ten days, the fungus degraded anionic surfactants continuously but in a lesser percentage. During the whole experimental period, the fungus removed 62.2% of anionic surfactant, *i.e.*, 620 μg mL^{-1}, in relation to the negative control (nkD5). The highest amount of removed surfactant the fungus utilized as source for biomass rebuilt. Due to this fact, a higher dry weight biomass (about 53%) was measured in medium D5 compared to the control. As seen in Figure 2, there is a strong linear relationship between the percentage of biodegradation of anionic surfactant and duration of experiment. Based on the equation of regression curve $y = 4.119x + 4.691$ (Figure 2), it would be predicted that the fungus would remove 80% of parent anionic surfactant of tested detergent in these experimental conditions for 18.28 days. These results indicate that anionic components of tested detergent satisfying required the limit of 80% biodegradability. According to Jerabkova *et al.* [20], *Pseudomonas* cultures in continuous bioreactors decomposed 70% of anionic surfactant after 20 days. However, Schleheck *et al.* [21] revealed that *Citrobacter* spp. has ability to degrade over 90% of anionic surfactant after 35 h of growth. Hosseini *et al.* [22] showed that *Acinetobacter johnsoni* strains can utilize 94% of the original SDS levels after 5 days. The results of these bacterial strains are far better in compare with our results but tested concentrations were lower than the applied concentration in this study. Also, pure anionic components are used for test of biodegradation by mention bacterial strain, whereas the commercial detergent used in this study is very complex. However, among numerous filamentous fungi species that were isolated from wastewater only the *M. racemosus* Fresenius had the ability to grow in a medium containing 0.5% detergent. This concentration exhibited a fungicidal effect on all the other tested fungi (unpublished date). Therefore, the biodegradation process is very complex and is dependent on numerous factors, such as type of surfactant and its concentration, microorganisms species, experimental conditions, availability of oxygen, etc. [23,24].

This study evaluated changes of pH and redox potential during fungal growth because these parameters are very important for regular growth and development and affect the morph-physiological characteristics and biochemical properties of 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 in the medium [25,26]. Therefore, the varied changes witnessed in the pH values of the culture media are a result of the utilization of nutrients from growth media [27]. Based on literature data, the fungus does not develop at pH above 9. This study provides evidence that *M. racemosus* Fresenius could tolerate wide range of environmental pH, from 4.75 to 9.80. Figure 3 shows that pH values of fermentation broth were changed during the growth of fungus 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 were changed in relation to their composition and growth phases of fungus. pH value of K medium was increasing from inoculation until 6th day. During stationary and autolysis phases, the pH value decreased slightly but these changes were not statistically significant. In contrast, the pH value of D5 medium was decreased in the exponential growth phase. The largest decrease of pH value was observed in medium D5 between the 3rd and 6th day (from 9.36 to 6.46 units) which corresponding to primary exponential growth phase. These changes of pH were expressed less in the secondary exponential growth phase. Interestingly, final pH values of different media were very similar beside the differences between the initial pH values were very significant.

Figure 4 illustrates that redox potential values of fermentation broth were 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 media before inoculation. One negative control (nkD5) with detergent but without spores was also

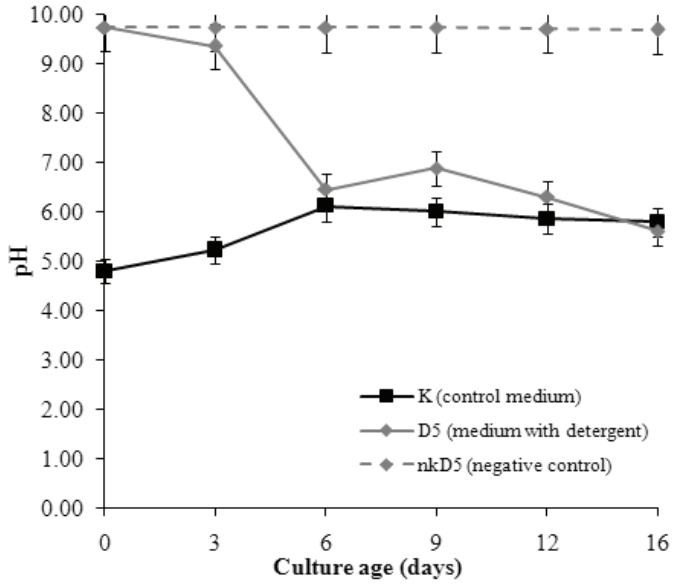

Figure 3. pH values of fermentation broth of K (control medium) and D5 (medium with 0.5% detergent) during fungal growth.

tested. The redox potential of K medium decreased during the exponential growth phase (from inoculation until 6th day), whereas it slightly increased throughout stationary and autolysis phases. During the biphasic exponential growth of fungus in D5 medium, the redox potential increased more intensively in the primary than in secondary exponential growth phase. The decrease in redox potential was measured in D5 medium on the 9th day only.

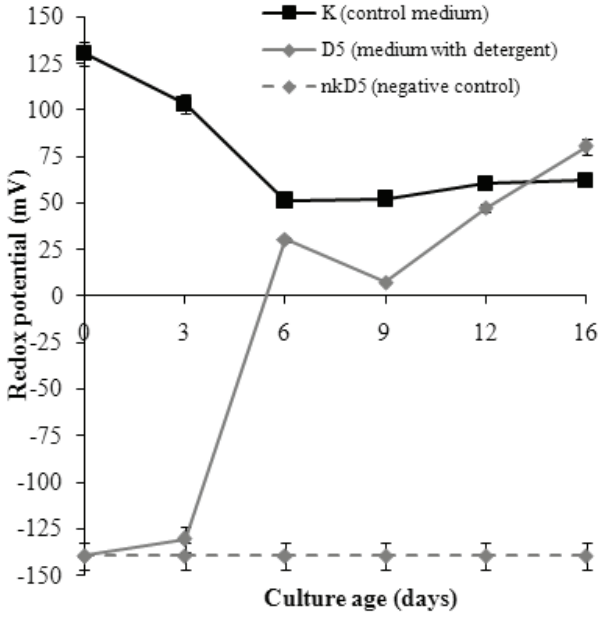

Figure 4. Redox potential values of fermentation broth K (control medium) and D5 (medium with 0.5% detergent) during growth of fungus.

Organic acids play a key role in alkali tolerance, especially for intracellular ionic homeostasis. Organic acids were produced by fungi into the medium can exist in free (FOA) and in bound form. The sum of amounts of both free and bound organic acids represents the amount of total organic acids (TOA). Figure 5 shows concentrations of free organic acids (FOA) and of total organic acids (TOA) in fermentation broth measured during the fungal growth. Concentration of free organic acids (FOA) in fermentation broth increased slowly or remarkably with culture age progression, depending on type of media. Amount of FOAs in K medium was in range from 0.04 to 0.06% during exponential phase, but when autolysis began, the amount of FOAs increased from 0.06 to 0.09%. Significant deviation of FOAs production was measured in D5 medium in relation to control. The concentration of FOAs increased from 0.02 to 0.06% in the primary exponential growth phase on the 6th day but the most significant increase was observed on 12th day in the secondary exponential growth phase from 0.06 to 0.12%. Maximal concentration of FOAs in detergent medium was about 50% higher compared to K medium (Figure 5). Concentration of total organic acids (TOAs) varied depending on the medium type and phases of fungal growth. In the early phase of fungal growth on 3rd day, concentration of TOAs was very uniform across the different media. The differences began manifesting on the 6th day, when higher amount of TOAs was observed in K medium (0.625%) than in D5 medium (0.4%). The concentration of TOAs increased negligibly in K medium whereas the concentration of TOAs decreased

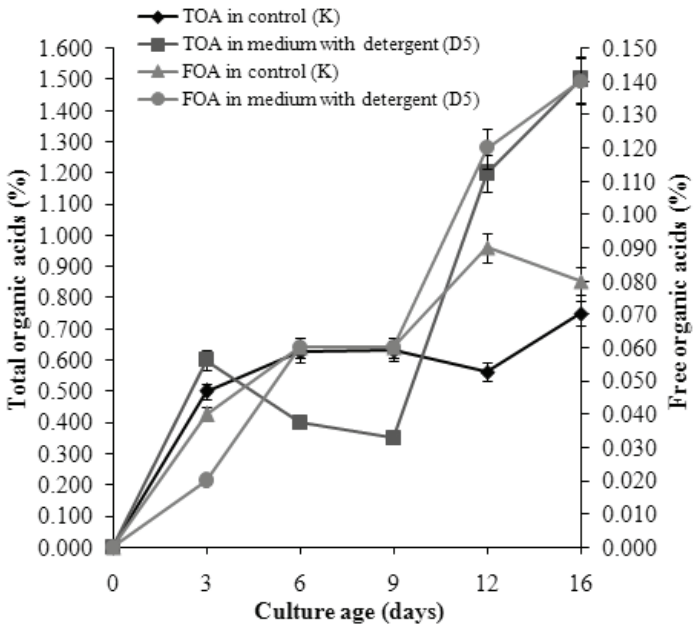

Figure 5. The concentrations of free (FOA) and total organic acids (TOA) measured in fermentation broth of K (control medium) and D5 (medium with 0.5% detergent).

negligibly in medium D5 until 9th day. Very significant increase in the concentration of TOAs was observed in D5 medium (from 0.35 to 1.5%) during fungal growth from the 9th to 16th day. The maximal amount of TOAs in detergent medium was higher in relation to the control (Figure 5). Statistical analysis of TOAs determined significant differences were between K and D5 media. This result could be explained by decrease of TOAs concentration in D5 medium from inoculation until the 9th day and its drastic increase from 9th to 16th day. The high correlation coefficient between concentrations of FOAs and TOAs $r = 0.771$, $p < 0.01$ suggests that amount of TOAs changes in towards increasing of FOAs rather than bound acids amount. Correlation coefficient between pH and FOAs $r = -0.517$, $p < 0.05$ indicates that strength rather than quantity of organic acids influence on pH value. There is a lot of evidence that the primary biodegradation begins with oxidation of the external methyl group (ω-oxidation) followed by stepwise shortening of the alkyl chain via oxidative cleavage of C2 units (β-oxidation). These processes lead to the formation of sulpho-phenyl carboxylic acids (SPACs) [28,29]. Wang *et al.* [30] reported that acetic, propionic, iso-valeric acids are dominant in process waste activated sludge digestion. The correlation between organic acids and fungal biomass indicates that some of organic acids mentioned above originate from detergent degradation ($r = 0.600$, $p < 0.01$).

Phosphatases represent a large group of enzymes acting on various phosphate esters. Phosphomonoesterase, the most studied group of phosphatases, hydrolyzes monoesters of phosphoric acid. Optimal pH value for activity of these enzymes is 9. Many alkaline phosphatases have a specific effect on substrate. This study revealed that *M. racemosus* Fresenius. has good capacity to produce alkaline phosphatase in K medium, as Figure 6 illustrates. The highest enzyme activity (73.23 IU mL^{-1}) in this medium was observed during exponential growth phase on the 6th day and was rapidly decreasing until the end of experiment. The maximal phosphatase activity was about 2.5 fold higher than phosphatase activity in medium with detergent. Because of specific effect of enzyme on β-glycerophosphate, higher enzyme activity is expected in K medium considering the composition of growth medium. Also, enzyme easily hydrolyzed monoesters that originated from carbohydrate metabolism than esters bonds in alkyl chain of surfactants. According to Koffi *et al.* [31], SDS display a strong inhibitory effect (about 98%) on phosphatase activity. The maximal value of phosphatase activity (27.478 IU mL^{-1}) in D5 medium was noted during autolysis phase. According to observed results, enzyme activity was inhibited by detergent (about 60%) in relation to the control.

CONCLUSIONS

This study claims that *Mucor racemosus* Fresenius is effective in biodegradation of anionic surfactant of tested commercial detergent in applied con-

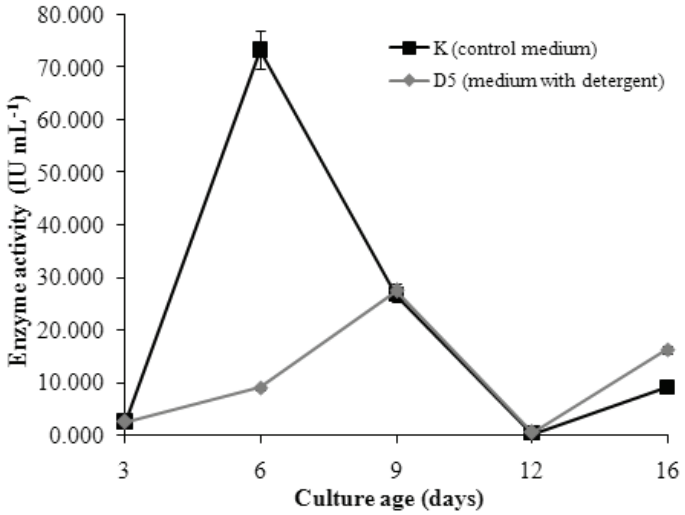

Figure 6. Profile of alkaline phosphatase activity of Mucor racemosus Fresenius cultivated in K (control medium) and D5 (medium with 0.5% detergent) during experimental period.

centrations, which is thousands folds higher than the law regulated concentrations that may be found in sewage water and natural recipients and lethal for the most microorganisms. In our opinion, this fungal resistance to the tested detergent could be explained by fungal morpho-anatomical characteristics as well as its physiological adaptation on presence of detergent in its habitat. The fungus could be successful applied in biological treatment of natural ecosystems as well as wastewater treatment plants. The results of this investigation showed that fungal alkaline phosphatase remained about 40% activity in the presence of detergent due to fungus could be applied in removing organic phosphates and decreasing eutrophication aquatic ecosystem.

Acknowledgments

This research was financially supported by Serbian Ministry of Education, Science and Technological Development (Grant number III 43004).

REFERENCES

[1] E.Z. Ron, E. Rosenberg, Curr. Opin. Biotechnol. **13** (2002) 249-252

[2] C.N. Mulligan, Environ. Pollut. **133** (2005) 183-198

[3] V. Chaturvedi, A. Kumar, IJABPT **1** (2010) 630-633

[4] I.M. Banat, R.S. Makkar, S.S. Cameotra, Appl. Microbiol. Biotechnol. **53** (2000) 495-508

[5] A. Petterson, M. Adamson, G. Dave, Chemosphere **41** (2000) 1611-1620.

[6] M. Raimbault, EJB Electronic. J. Biotech. **1** (1998) 174--188

[7] European Parliament Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents, In Official Journal of the European Union, EC No 684/2004, 2004

[8] V.G. Lilly, H.L. Barnett, Physiology of the fungi, McGraw--Hill Book Co., New York, 1951, p. 464

[9] P. Borgia, P.S. Sypherd, J. Bacteriol. **130** (1977) 812-817

[10] APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, D.C., 1989, p. 1268

[11] W.A. Bulen, J.E. Varner, R.C. Burrell, Anal. Chem. **24** (1952) 187-190

[12] M. Somogyi, J. Biol. Chem. **195** (1952) 19-23

[13] J.K. Heinonen, R.J. Lahti, Anal. Biochem. **113** (1981) 313-317

[14] J. Mehta, M. Jakhetia, S. Choudhary, J. Mirza, D. Sharma, P. Khatri, Eur. J. Exp. Biol. **2** (2012) 2061-2067

[15] A. Peksel, C.P. Kubicek, Turk. J. Chem. **27** (2003) 581--590

[16] Z. Barboráková, R. Labuda, G. Häubl, D. Tančinová, JMBFS **1** (2012) 466-477

[17] M. Velan, S. SheebaVarma, P. Gnanambigai, M.B. Lakshmi, IJCEES **3** (2012) 318-323

[18] D. Schleheck, A.M. Cook, Arch. Microbiol. **183** (2005) 369-377

[19] A.Y. Kagalwala, K. Kavitha, IJLBPR **1** (2012) 128-138

[20] H. Jerabkova, B. Kralova, J. Nahlik, Int. Biodeteriorat. Biodegradat. **44** (1999) 233-241

[21] D. Schleheck, M. Lechner, R. Schonenberger, Appl. Environ. Microb. **69** (2003) 938-944

[22] F. Hosseini, F. Malekzadeh, N. Amirmozafari, N. Ghaemi, Int. J. Environ. Sci. Tech. **4** (2007) 127-132

[23] F. Ahimou, P. Jacques, M. Deleu, Enzyme Microb. Technol. **27** (2000) 749-754

[24] H. Zhong, G.M. Zeng, J.X Liu, X.M. Xu, X.Z. Yuan, H.Y. Fu, G.H. Huang, Z.F. Liu, Y. Ding, Appl. Microbiol. Biotechnol. **79** (2008) 671-677

[25] E. Moore-Landecker, Fundamentals of Fungi, Prentice Hall, Upper Saddle River, New York (1996), p.574

[26] D.H. Griffin, Fungi Physiology, Wiley-Liss, New York 1994, p.468

[27] M. Orlowski, Microbiol. Rev. **55** (1991) 234-258

[28] A.M. Cook, Tenside Surfact. Det. **35** (1998) 52-56

[29] J.D. Van Hamme, A. Singh, O.P. Ward, Microbiol. Mol. Biol. Rev. **67** (2003) 503-549

[30] Q.H. Wang, M. Kuninobu, H.I. Ogawa, Y. Kato, Biomass Bioenerg. **16** (1999) 407-416

[31] D. Koffi., B. Faulet, J. Gonnety, M. Bédikou, L.P. Kouame, I.A. Zoro Bi, S.L. Niamke, Sci. Nat. **7** (2010) 221--235.

VIOLETA D JAKOVLJEVIĆ[1]
JASMINA M MILIĆEVIĆ[1]
JELICA D STOJANOVIĆ[1]
MIROSLAV M VRVIĆ[2]

[1]Institut za biologiju I ekologiju, Prirodno-matematički fakultet, Univerzitet u Kragujevcu, Kragujevac, Srbija
[2]Katedra za biohemiju, Hemijski fakultet, Univerzitet u Beogradu, Beograd, Srbija

NAUČNI RAD

SPOSOBNOST GLJIVE *Mucor racemosus* FRESENIUS DA RAZLAŽE DETERGENT U VELIKOJ KONCENTRACIJI

Sposobnost gljive Mucor racemosus Fresenius da razlaže komercijalni detergent (Merix, Henkel, Srbija) u velikoj koncentraciji ispitivana je u ovom radu. Gljiva je gajena u tečnoj hranljivoj podlozi po Čapeku sa dodatkom detergenta 0,5% koncentracije, u vremenskom periodu od 16 dana. Biohemijske promene: pH, redoks potencijal, količina slobodnih i ukupnih organskih kiselina i aktivnost alkaline fosfataze, ispitivane su analizom fermentacione tečnosti. Procenat biodegradacije anjonske komponente detergenta određen je primenom MBAS metode. Istovremeno, analiziran je uticaj detergenta na rast i ukupnu suvu biomasu gljive. Detergent 0,5% koncentracije uticao je na smanjenje pH vrednosti i povećanje redoks potencijala, kao i na povećanje količine slobodnih i ukupnih organskih kiselina. Aktivnost alkaline fosfataze bila je smanjena u podlozi sa 0,5% detergenta. Gljiva je razgradila oko 62% testiranog detergenta tokom ogledog perioda od 16 dana. Kao rezultat degradacije detergenta, gljiva je produkovala veću količinu ukupne suve biomase (53%) u odnosu na kontrolu.

Ključne reči: biomasa, biodegradacija detergenta, enzimska aktivnost, organske kiseline, pH, redoks potencijal.