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THE POTENTIAL APPLICATION OF FUNGUS *Trichoderma harzianum* RIFAI IN BIODEGRADATION OF DETERGENT AND INDUSTRY

Article Highlights

- *T. harzianum* isolated from industrial wastewater was tested to biodegradation of detergent
- Chemical and biochemical parameters examined during fungal growth in submerged fermentation
- The fungus decomposed 74.24% of detergent during 16 days which was confirmed by MBAS assay
- Alkaline protease activity in presence of detergent was enhanced by 128% compared to control

Abstract

The potential application of fungus Trichoderma harzianum Rifai in biodegradation of commercial detergent (Merix, Henkel, Serbia) was the focus of this study. The fungus was isolated from wastewater samples of the Rasina River, downstream from where the industrial wastewaters of the plant Henkel (Kruševac, Serbia) discharge into the river. The fungus was cultivated in liquid growth medium by Czapek with addition of detergent at a concentration of 0.3% during 16 days. Analysis of fermentation broth evaluated the chemical and biochemical changes of pH, redox potential, activity of alkaline and acid invertase as well as activity of alkaline protease. In addition, the influence of detergent on fungal growth and total dry weight biomass was determined. At the same time, detergent disappearance in terms of methylene blue active substances in the medium was measured. The detergent at a concentration of 0.3% influenced significant decrease of pH value and increase of redox potential. The detergent showed inhibitory effect on acid invertase activity and stimulatory effect on alkaline invertase and protease activity. The fungus decomposed about 74.24% of tested detergent during 16 days, but total dry weight biomass reduced about 20% in relation to control.

Keywords: alkaline protease activity, invertase activity, biomass, biodegradation, pH, redox potential.

Detergents are very widely used in both industrial and domestic premises like soaps and detergents to wash vehicles, in pesticide formulations and for dispersing oil spills at sea [1]. Consequently, a large quantity of detergent can be expected in the environment. The major entry point into water is via sewage works into surface water. The toxic effects of surfactants are well studied. The ability of surfactants to absorb and penetrate into cell membranes of aquatic organisms determined the degree of surfactants toxicity [2]. The effect of surfactants on bacteria reflects in the inhibition of cell proliferation, and reducing the degradation ability of PAHs (polycyclic aromatic hydrocarbons). Toxic molecules of surfactant induce apoptosis or necrosis of cells, depending on the concentration of surfactants. The toxicity of surfactants depends on their molecular structure. Generally, nonionic surfactants are less toxic than the ionic surfactants. According to the literature, the anionic surf-

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actants have a toxic effect on a variety of aquatic organisms in concentrations below 0.0025 mg L^{-1} [3]. Many researchers revealed that the surfactant changes the conformation of the protein and thus affect the enzyme activity, stability, and specificity [4,5]. As a response to the presence of various pollutants that disrupt natural ecosystems, a number of physical and chemical methods have been developed for the removal of xenobiotics from the environment [6]. Using the ability of microorganisms, which are able to exploit a wide range of compounds as carbon and energy sources, for the purpose of purification of the pollutants is referred to as bioremediation and represents a promising, relatively efficient and very expensive technology [7]. A large number of species of microorganisms (bacteria, fungi and algae) has been identified as capable for biodegradation the organic compounds that have reached the environment by human action. Filamentous fungi have the potential to grow in the presence of various pollutants, which are degraded to simpler molecules that can be used as a carbon and energy source [8]. In addition, the fungi have a characteristic ability to produce a large number of extracellular proteins, organic acids and other secondary metabolites, due to adaptation to changing environmental conditions. Recently, it was confirmed that *M. racemosus* can degrade a high concentration of commercial detergent (0.5%) [9], whereas *P. chrysogenum* can degrade lower detergent concentration (0.3%) [10].

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 [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 the 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.

There is no published data related to testing the ability of detergent biodegradation by *T. harzianum*. Therefore, the aim of this study was to investigate the ability of *T. harzianum* to degrade of commercial detergent, which is very significant water pollutant, in the purpose of its potential use in bioremediation. In addition, the influence of detergent on the growth, protease and invertase activity of fungus was in the focus of this research, in order to investigate the potential use of the enzymes produced by the fungus in biotechnology and detergent industry.

EXPERIMENTAL

Isolation and identification of *Trichoderma harzianum* Rifai

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

Fermentation 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 to1000 mL (control-C) and the same medium with additional 3 g of detergent to obtain concentration of 0.3% (medium D3).

Erlenmeyer flasks with liquid growth medium (200 mL of medium in 250 mL flask) 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 L^{-1} HCl. After addition of detergent to liquid growth medium pH value of 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 was 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) 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 10000*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/temperature meter) type of Bante with glass electrode model 65-1.

Determination of dry weight biomass

The dry weight biomass of the mycelia was determined according to procedure as described in our previous work [9].

Determination of anionic surfactant and biodegradation rate

The concentration of anionic surfactant was determined by spectrophotometric methods using methylene blue. The method for determining the concentration of methylene blue-active substance (MBAS) in the detergents was adapted from Standard Methods for the Examination of Water and Wastewater [14] as described in our previous work [9]. The percentage of degradation was then calculated as:

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

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

Assays of acid and alkaline invertase activity (EC 3.2.1.26)

Alkaline invertase activity was assayed in a reaction mixture that consisted of 0.06 mol·L^{-1} sucrose, 0.02 mol·L^{-1} citrate buffer (pH 8.0) and 300 µL of enzyme solution, in a final volume of 1 mL. The mixture was incubated at 37 ºC for 30 min. The reaction mixture for acid invertase activity contained the same components, except that citrate buffer was replaced with 0.1 mol·L^{-1} sodium acetate buffer (pH 4.5). The reaction mixture was incubated at 55 °C for 30 min. The amount of reducing sugar liberated was determined spectrophotometrically at 410 nm, by the method of Somogyi-Nelson [15]. One unit of invertase activity (IU) was defined as the amount of enzyme that catalyzed the production of 1 µmol of glucose per min at 37 °C.

Assays of alkaline protease activity (EC 3.4.21-24)

Activity of alkaline protease was determined spectrophotometrically by Anson's method, with casein as substrate [16]. Reaction mixture incubated at 37 °C for 10 min and arrested by addition of 1 mL 5% trichloroacetic acid (TCA). The mixture was centrifuged at 4000*g* min^{-1} and to the supernatant 5 mL of 6% Na_2CO_3 and 1mL diluted Folin-Ciocalteu's phenol reagent were added. The resulting solution was incubated at room temperature for 30 min and absorbance of the blue color developed was read at 660 nm using tyrosine standard. One unit enzyme activity (IU) was defined as the amount of enzyme that liberated 1 µg of tyrosine from casein per minute under assay condition.

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 growth of fungi is conditioned by numerous factors: pH, aeration, temperature, chemicals, *etc*. Czapek-Dox medium has good properties for growth and high biomass production of the most fungi [17,18]. The presence of detergent in the culture medium affects the fungal biomass production according to the type of detergent, fungi species and the applied concentration, due to the influence of detergent on the growth and total dry weight biomass of fungus *T. harzianum* Rifai investigated in this study, as shown in Figure 1.

Our results showed that the fungus cultivated in the control medium (C) had a monophasic exponential growth from the inoculation until the 6th day. From the 6th day to 9th day, there was stationary phase when the maximal dry weight of biomass was achieved. After stationary phase, autolysis and reduction of biomass began on the 12th day. These results are in accordance with results of other authors [19,20]. The same fungus cultivated in medium with 0.3% detergent (D3), showed biphasic exponential growth with distinct two stationary phases and the absence of autolysis. The early phase of fungal growth was manifested from the inoculation until 3rd day followed by the first stationary phase (from 3rd to

6th day). Exponential growth of fungus was continued from the 6th day to the 9th day, when statistically significant increase of biomass was observed. The second stationary phase began on the 9th day and lasted until the 16th day, when a maximal dry weight biomass was obtained. However, the dry weight of biomass was slightly lower in a medium D3 (1.015 g L^{-1}) in comparison to C medium (1.269 g L^{-1}) on the 16th day. The detergent added in growth medium in concentration of 0.3% inhibited about 20% of the dry weight biomass of *T. harzianum*, probably due to toxic effects some degradation products on fungal growth. It is well known that detergent has inhibitory effect on enzymes involve in key metabolic pathways. Also, physico-chemical interactions between surfactants and fungal structures such as membranes and walls influence on fungal growth [21]. Many investigations confirm growth inhibition by the anionic surfactant, SDS [22]. The results obtained in this study agree with the results of some researchers and our results obtained for *P. chrysogenum*, but are contrary to results obtained for *M. racemosus*. Czapek Dox liquid medium with addition of detergent at concentration of 0.5% strongly stimulated of total biomass production of *M. racemosus* in the same experimental conditions. Since both *M. racemosus* and *T. harzianum* were isolated from the same locality, it would be expected that both species successfully break down detergent at concentrations of 0.5%. However, this concentration of detergent expressed a fungicide effect on *T. harzianum*. It is obvious that the differences in ability of biodegradation of detergent can be attributed to morpho-physiological differences of fungi.

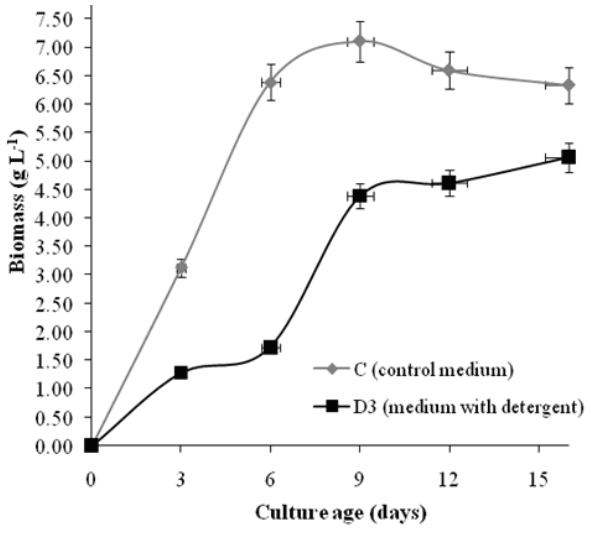

Figure 1. Display of growth and total dry weight biomass of fungus Trichoderma harzianum Rifai cultivated in C (control medium) and D3 (medium with 0.3% detergent) during experimental period.

The biodegradation rate of tested detergent by *T. harzianum* is shown in Figure 2. The percentage of biodegradation of detergent was examined in inoculated medium with detergent (D3) during the experimental period. Non-inoculated medium with detergent (ncD3) was used as the negative control and showed that the tested detergent was chemically stable in the experimental conditions. The initial concentration of detergent in the medium D3 decreased continuously with the growth and development of the mycelium, so it reduced from 3 to 0.811 mg mL^{-1} for 16 days. The fungus decomposed 74.27% of the total detergent concentration in the medium at the end of the experimental period. During the first 3 days of the fungal growth, it degraded 17.01% of detergent whereas in the period from the 3rd day until the 6th day the fungus degraded total 25.62% of detergent. A significant increase of the biodegradation rate observed in the exponential growth phase, from the 6th day to the 9th day, during which the fungus degraded total 62.23% of tested detergent. The biodegradation rate was very slightly increasing with aging of fungus and ranged in the next direction: from the 9th day to the 12th day it was 65.87%; from the 12th day to the 16th day 74.27%. A very high correlation coefficient was found between biodegradation rate and biomass (r = 0.999, p < 0.01) as well as between duration of experiment and biodegradation rate (r = 0.936, p < 0.05). These results are agreement with results of some authors who confirmed that degradation rate of surfactant is concomitants with cellular growth [23]. The tested detergent contains about 20% anionic surfactant, which is confirmed by MBAS assay. When the concentration of anionic surfactant was expressed in μg mL^{-1} it was obtained that initial concentration of anionic surfactant was 600 μg mL^{-1}. Based on these results, fungus decomposed 454.6 μg mL^{-1} anionic surfactant of detergent at the end of the experimental period. From the equation of regression curve (y = = 4.898x + 2.600), it would be predicted that the fungus will remove 80% of parent anionic surfactant of tested detergent in these experimental conditions for 16.8 days. This result is very similar to result obtained in biodegradation test by *M. racemosus* in the same conditions [9]. However, in the literature can be found very little data about biodegradation of detergent by fungi, in contrast to bacteria, which are well studied as test organisms. Jerabkova *et al.* [24] revealed that *Pseudomonas* cultures in continuous bioreactors decomposed 70% of anionic surfactant after 20 days; whereas Schleheck *et al.* [25] revealed that *Citrobacter* spp. have ability to degrade over 90% of anionic surfactant after 35 h of growth. Results of

Hosseini *et al.* [26] showed that *Acinetobacter johnsoni* strains could utilize 94% of the original SDS levels after 5 days. It is important to point out that the biodegradation percentage observed by mention bacterial strains is far better than achieved in this study but the concentration tested in this study was far higher. In addition, the commercial detergent used in this study is very complex and thus any individual component can interfere with biodegradation, whereas the pure anionic components used for test of biodegradation by mention bacterial strain.

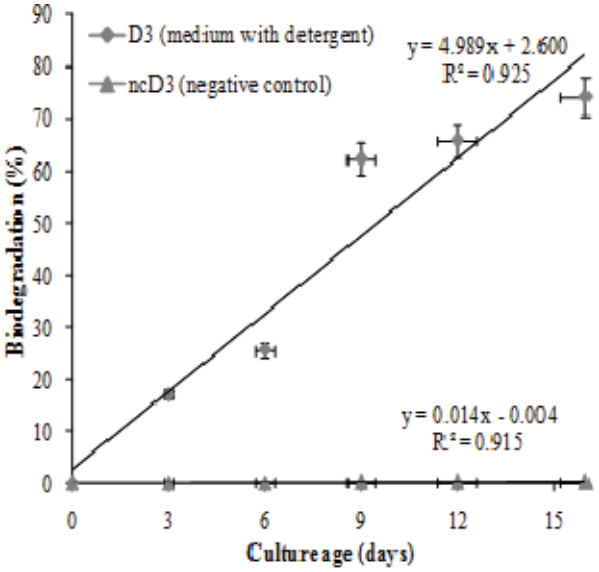

Figure 2. Percentage of biodegradation of anionic surfactants of detergent measured in fermentation broth of D3 (medium with 0.3% detergent) compared to negative control (ncD3).

The pH and redox potential values are very important parameters for regular growth and development, and influence on the morpho-physiological characteristics and biochemical properties of micro-organisms. In addition, it has application in the monitoring of bioremediation processes. The optimum external pH for fungal growth is located in acidic range, from 4.5 to 5. Generally, fungi alter the pH of the medium in which they grow, due to uptake of the anions or cations in the medium [27,28]. Therefore, the changes in the pH values of the culture media are a result of the utilization of nutrients from growth media [29].

The changes of pH values of the fermentation broth during the growth and development of the mycelium of *T. harzianum* from inoculation until the 16th day are shown in Figure 3. The pH value of fermentation broth was measured in the control medium (C), as well as in inoculated (D3) and non-inoculated (ncD3) detergent media. The initial pH values media were 4.75 in C, and 9.35 in D3 (ncD3) media. The pH values of inoculated growth media were changes in relation to their composition and fungal growth phases. The significant changes of the pH values of C medium observed during the phase of exponential growth of fungus. The pH values of this medium increased during the first 3 days (from 4.80 to 5.40 units), and then decreased in the period from the 3rd day to the 6th day (from 5.40 to 4.82 units). Insignificant changes of pH values were noted during the stationary phase and autolysis. The pH value of fermentation broth of D3 medium decreased throughout the experimental period. There was a slight change of pH value in the initial phase of fungal development, from inoculation until 3th day. However, the most significant decreasing of pH value (from 9.05 to 6.07 units) was observed from the 3th day to the 6th day. High correlation coefficient was found between pH value and biomass (r = -0.813, p < 0.01).

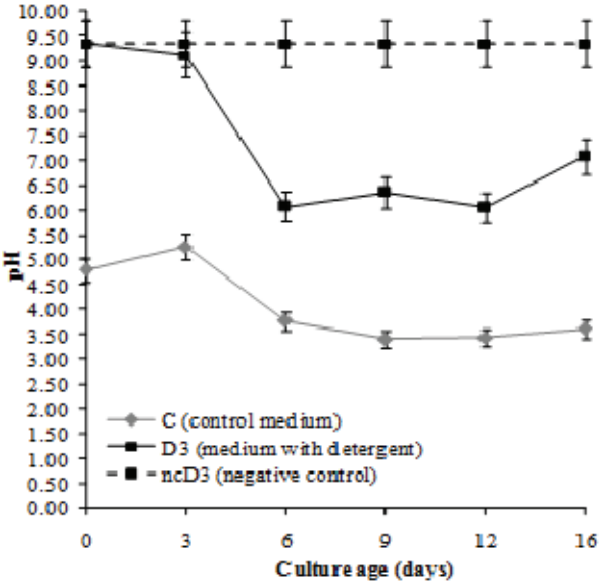

Figure 3. pH values of fermentation broth of C (control medium) and D3 (medium with 0.3% detergent) during fungal growth compared to negative control (ncD3).

Figure 4 shows that redox potential values of fermentation broth changed during the growth of fungus *T. harzianum* in a control medium (C), as well as in inoculated detergent medium (D3). A negative non-inoculated control with detergent (ncD3) was also tested and during the experimental period, no changes in its redox potential value were observed. The initial redox potential values were 340 mV in C and 80 mV in D3 (ncD3) media. The significant changes of redox potential observed during the first 6 days of fungal growth in the control medium (C). The redox potential of this medium decreased rapidly during the first 3 days (from 340 to 274 mV), but then

increased in the period from the 3rd day to the 6th day (from 274 to 290 mV). During the stationary and autolysis phase, the redox potential values decreased continuously. The redox potential of D3 medium increased throughout the experiment. The redox potential value increased slightly during the first 6 days, whereas the significant changes of redox potential value (from 97 to 292 mV) observed during the exponential growth of the fungus (from 6th day to 9th day). During stationary phase, the changes of redox potential values were insignificant. High correlation coefficient was found between redox potential value and biomass (r = -0.675, p < 0.05).

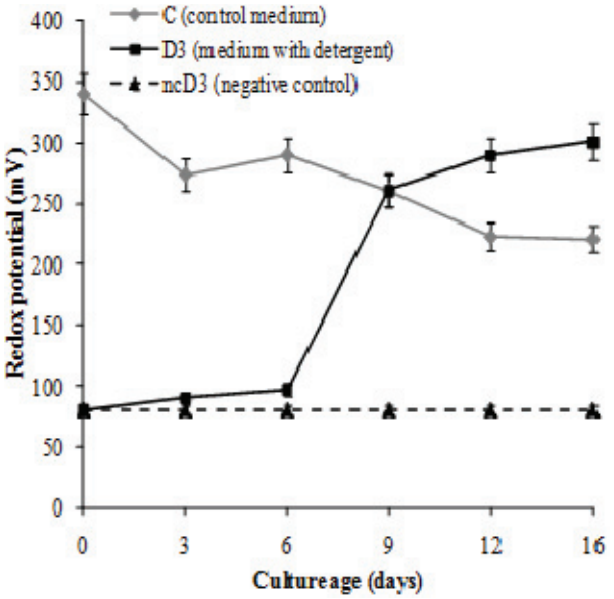

Figure 4. Redox potential values of fermentation broth of C (control medium) and D3 (medium with 0.3% detergent) during fungal growth compared to negative control (ncD3).

Invertase is a key metabolic enzyme that hydrolyzes the disaccharide sucrose to glucose and fructose. This enzyme finds numerous applications in the food industry. Confectionary's preference for invert sugar hovers around its ability to keep the products fresh and soft for prolonged periods. Soluble invertase is used in the sweet industry for the production of artificial honey. The presence of sugar (mostly glucose) in the nutrient medium is necessary for the biodegradation of the surfactant by fungus. The production of invertase from *Penicillium* sp. and *Aspergillus* sp. has been reported [30-32]. According to Poonawalla *et al.* [33], the fungus *P. chrysogenum* has both extracellular and intracellular invertase, which are distinguished by their subcellular localization (cell wall, vacuole or cytosol), solubility (soluble or insoluble in low ionic strength buffer), optimum pH (acid or neutral/alkaline) and isoelectric point (p*I*).

Figure 5 shows the activity of acidic and alkaline invertase of the fungus *T. harzianum* during fungal growth in the control (C) and detergent inoculated (D3) media. Acid invertase activity (AcI) of fungus was weak in the initial stage of mycelial growth from the 3rd day to the 6th day, but then increased rapidly in C medium during the period from the 6th day to the 9th day, when it achieved a maximum value (0.08 IU mL^{-1}). The activity of AcI decreased rapidly followed by a period from the 9th day to the 12th day, when was totally inhibited in C medium. However, acid invertase activity was very weak in D3 medium and expressed only in the phase of growth of the mycelium from the 3rd day to the 6th day. Alkaline invertase activity (AlkI) in C medium was measured in the early phases of growth of mycelium, with the maximum achieved on the 3rd day, but decreasing until the 16th day. In the medium D3, activity of AlkI was negligible until the 6th day, and then enzyme activity increased rapidly with the maximum achieved on the 12th day. The obtained results confirmed that the activity of these enzymes depends on the type of media and the stage of fungal development Therefore, acid invertase activity was higher than alkaline invertase activity in C medium, whereas the alkaline invertase activity was higher than acid invertase activity in D3 medium. Finally, our results strongly support that fungus can be used for the commercial preparation of invertase in acid and alkaline pH conditions.

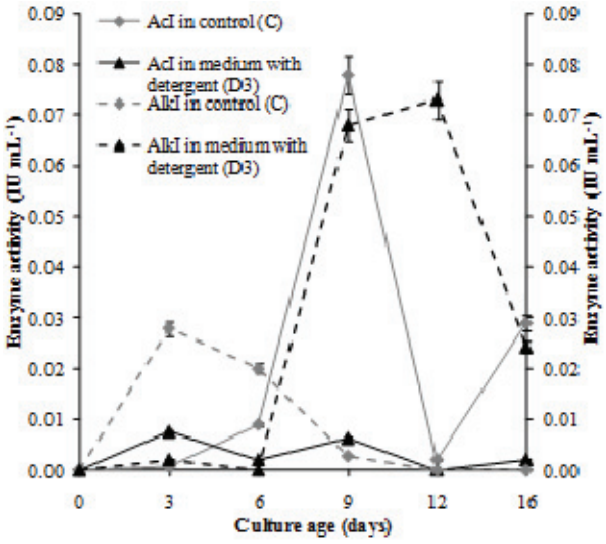

Figure 5. Profile of alkaline and acid invertase activity of fermentation broth Trichoderma harzianum Rifai cultivated in C (control medium) and D3 (medium with 0.3% detergent) during experimental period.

Alkaline proteases improve the cleaning efficiency of detergents and represent one of the most successful applications of modern industrial biotechnology [34]. Enzyme stability in the presence of detergent ingredients, such as surfactants, builders and activated bleach, etc. is crucial for its use in detergent formulations [35]. The proteolytic activity of different species filamentous fungi in different growth medium is well studied. Studies on fungi have shown that, in most cases, proteolytic activity increases with the beginning of autolysis, or maximum proteolytic activity to coincide with the growth phase. Delgado-Jarana *et al.* [36] detected several acidic, neutral and basic extracellular proteases in the case of *T. harzianum*. A role of the enzymes produced by the mycoparasitic fungi *Trichoderma* in biological control of fungal pathogens like *Botrytis* is documented. Recently, extracellular serine protease produced by *T. harzianum* originated from a sago industry was isolated [37]. The protease *T. harzianum* had optimum pH and temperature of 7.0–8.0 and 50–60 °C, respectively. At 37 °C and 60 °C, the parental proteases were respectively stable for 1 day and 15 min. The fungal enzyme retained maximum residual activity of 64 and 55% with the commercial detergent Rin Advanced and Kite, respectively. In the literature, there is much evidence about the effects of anionic surfactants (*e.g.* SDS) on enzyme activity of some *Bacillus* species. Protease from thermophilic *Bacillus* sp. retained more than 80% and 65% of its activity after 30 min incubation at 60 °C in the presence of the detergent brands Tide® and Cheer®, respectively [20]. The protease from *Bacillus brevis* showed compatibility at 60 °C with commercial detergents such as Ariel®, Surf Excels®, Surf Ultra® and Rin® in the presence of Ca^{2+} and glycine. According to Subba Rao *et al.* [38], in the presence of 1% strong anionic surfactant SDS, the enzyme retained 75% of its initial activity.

In this study, proteolytic activity of the fungus *T. harzianum* during fungal growth in the control (C) and detergent inoculated (D3) media observed. The results are shown in Figure 6. The proteolytic activity of the fermentation broth the fungus *T. harzianum* in C medium was expressed only in early phase of mycelial development (3rd day to 6th day), when the maximal enzyme activity was observed (0.274 IU mL^{-1}). With the beginning of the stationary phase, enzyme activity rapidly decreased and the lowest activity was observed during autolysis (12th day). In the medium D3, proteolytic activity was stimulated by the presence of tested detergent compared to the control. The maximum enzyme activity was observed at the end of the first stationary phase and with beginning exponential growth phase on the 6th day (0.5943 IU mL^{-1}) as well as in the second stationary phase on 12th day (0.625 IU mL^{-1}). Minimum proteolytic activity measured on the 16th day in both types of media. In brief, tested anionic detergent enhances proteolytic activity of fungus for 128% in relation to control. Based on the presented results, the performance of alkaline protease of fungus *T. harzianum* is suitable for potential application as additive in laundry detergent formulations.

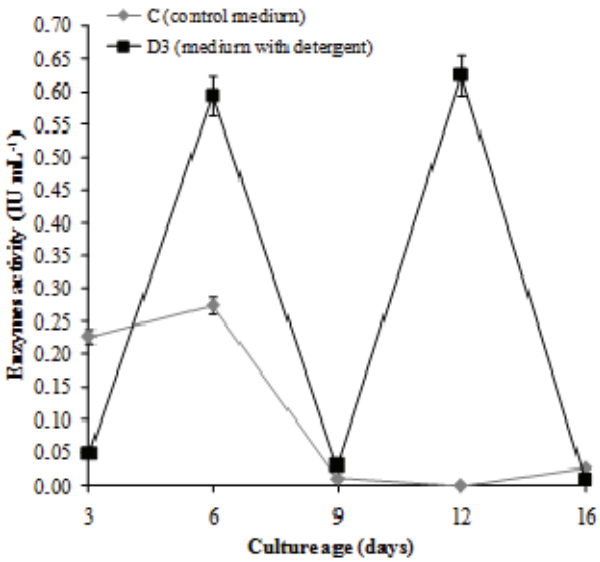

Figure 6. Profile of alkaline protease activity of fermentation broth of Trichoderma harzianum Rifai cultivated in C (control medium) and D3 (medium with 0.3% detergent) during experimental period.

CONCLUSION

The presented results indicate that *Trichoderma harzianum* Rifai can be characterized as a promising detergent degrading strain. In applied experimental conditions, the fungus degraded total 74.24% of tested commercial detergent or 454.6 μg mL^{-1} anionic surfactant of detergent during 16 days. According to this data, the fungus could be successfully applied in biological treatment of natural ecosystems, as well as wastewater treatment plants. The results of the investigation of acid and alkaline invertase activity indicate that the fungus can be exploited as a source of invertase in acid and alkaline pH conditions. The results also showed that activity of fungal alkaline protease in presence of tested detergent was enhanced about 128% compared to control. Therefore, this fungus species could be used for production of alkaline protease with performance suitable as detergent additive.

Acknowledgments

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NAUČNI RAD

POTENCIJALNA PRIMENA GLJIVE *Trichoderma harzianum* RIFAI U BIODEGRADACIJI DETERGENTA I INDUSTRIJI

Potencijalna primena gljive Trichoderma harzianum Rifai u biodegradaciji komercijalnog detergenta (MERIX, Henkel, Srbija) bila je predment ovog istraživanja. Gljiva je izolovana iz uzoraka otpadnih voda reke Rasine, nizvodno od mesta gde se industrijske otpadne vode fabrike Henkel (Kruševac, Srbija) izlivaju u reku. Gljiva je gajena u tečnoj hranljivoj podlozi po Čapeku sa dodatkom detergenta 0,3% koncentracije, u periodu od 16 dana. Analizom fermentacione tečnosti ispitivane su hemijske i biohemijske promene: pH, redoks potencijal, aktivnost alkalne i kisele invertaze i alkalne proteaze. Takođe, ispitivan je uticaj detergenta na rast gljive i ukupnu suvu biomasu. Istovremeno, smanjenje koncentracije detergenta u hranljivoj podlozi mereno je MBAS metodom. Detergent 0,3% koncentracije uticao je na značajno smanjenje pH vrednosti i povećanje redoks potencijala fermentacione tečnosti. Detergent je ispoljio inhibitorno dejstvo na aktivnost kisele invertaze i stimulativno dejstvo na aktivnost alkalne invertaze i proteaze. Tokom 16-todnevnog eksperimentalnog perioda gljiva je razgradila oko 74,24% testiranog detergenta, a ukupna suva biomasa bila je redukovana oko 20% u odnosu na kontrolu.

Ključne reči: aktivnost alkalne proteaze, aktivnost invertaze, biomasa, biodegradacija, pH, redoks potencijal.