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Influence of detergent on metabolic activity of fungi *Aspergillus niger*

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ABSTRACT

The aim of this study was to find out, among grate variety of fungi species from wastewater these which are resistant to effects of detergent and its component, ethoxyl-oleyl-cetyl alcohol and sodium tripolyphosphate. On inoculated fungi specie grown *in vitro* condition, in the presence of mentioned pollutant, the metabolic changes of bioproduction of different organic compounds, in various aging step of fungi, have been investigated. The results indicated significant changes in bioproduction of amino acids and proteins of *Aspergillus niger* cultivated in the presence of detergent and its component, compared with control experiment. The results suggest that bioremediation by *Aspergillus niger* are promising for biodegradation of detergents in aquatic systems.

Keywords: Aspergillus Niger; *Detergent*; *Bioproduction*; *Wastewater*; *Biodegradation*

1. INTRODUCTION

Detergents are formulations designed to have cleaning/solubilisation properties. These formulations consist of surface-active agents (surfactants) together with subsidiary components including builders (e.g. tripolyphosphate), boosters, filters and auxiliary compounds. Surfactants are a group of organic compounds achieved by chemical synthesis and characterized for specific behavior in solution that makes them especially suitable for many human activities. Surfactant is an abbreviation for a surface-active agent that refers to its ability to reduce the interfacial tension between two phases. This behavior is caused by the molecular composition in the surfactant, which has a hydrophobic part, composed of alkyl chains, and another part that is an anionic or hydrophilic group [1].

A massive stream of surfactants is directed to the aquatic environment. Surfactants are probably the largest supplier of artificial organic carbon to the aquatic environment [2]. Even though surfactants are essentially non-toxic to man at the concentrations likely to be met in wastewaters, there is wide agreement that their presence both in natural freshwater sources and in other ecosystems is undesirable. The principal criterion for the ecological behavior of surfactant is their biodegradability [3].

Many researchers fortified that microorganisms, particularly some kind of fungi, can act as potential degraders of detergents [4,5]. Among the fungi which have such ability, filamentous fungi (*Deuteromicotina)* are especially distinguished due to their physiological and biochemical characteristics [6]. 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 which fungi can use for growth and development of mycelia and biomass accumulation [7-9].

The *Aspergillus* fungus was first recognized as an organism in 1729 by Micheli [10]. 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 [11]. Although it includes the major filamentous fungal pathogen of humans, *Aspergillus fumigatus* [12], most of the members are useful microorganisms in nature for degradation of plant polysaccharides [13], and they are important industrial microorganisms for the large-scale production of both homologous and heterologous enzymes [14-18]. Among them, *Aspergillus oryzae* and *Aspergillus niger* are on the Generally Recognized as Safe list of Food and Drug Administration in the United

States [19]. *Aspergillus niger* is one of the most important microorganisms used in biotechnology [20,21] which produces many extracellular enzymes.

Detergents have severe effects on wildlife and human health due to their toxicological properties [22]. Linear alkylbenzene sulphonate (LAS) is anionic surfactant most widely used as a major ingredient in domestic and industrial detergents. It can easily be degraded by microorganisms in wastewater treatment plants using aerobic processes [23,24], however the intermediates are less biodegradable [25]. Such metabolites may be toxic to higher biotrophic members of the ecosystem, but they also offer a potential carbon and energy source for microorganisms capable of breaking them down further. LAS degradation may be performed by specific microorganisms that use it as a sole carbon source [26] or as a co-metabolic transformation [27]. Balson and Felix [28] described biodegradation as the destruction of a chemical by the metabolic activity of microorganisms. When reviewing the literature concerning the degradation of surfactants it is apparent that studies quote figures for primary and/or ultimate biodegradation [29]. Primary degradation can be defined as to have occurred when the structure has change sufficiently for a molecule to lose its surfactant properties. Ultimate degradation is said to have occurred when a surfactant molecule has been rendered to CO_2, CH_4, water, mineral salts and biomass. Primary degradation of LAS on activated sludge is grater then 99% [30-32]. Traces of LAS in natural waters and soil continuo rapid degradation (half-live of LAS is about 0.15-0.5 days), but total biodegradation still requires several days [33-37].

Surfactants and water miscible organic solvents are frequently used to increase the bioavailability of environmental contaminants for degradation [38]. LAS is used as a mediator in polyaromatic hydrocarbons degradation catalyzed by the extracellular enzyme system of some fungi [39]. Also, it was found that addition of detergents have enhancing effects on extracellular production of some metabolites with microorganisms [40] and may be useful method for over-production of hydrophobic compounds by means of biological process.

Herein we describe our studies on influence of detergent on metabolic activity of fungi *Aspergillus niger*.

2. MATERIAL AND METHODES

The experiments were performed using monosporial culture of the fungi *Aspergillus niger* van Tiegheme isolated from the river Lepenica (Serbia) on wastewater outpouring site. Identification of the culture was done on Faculty of biology, Belgrade, in Laboratory for algae, fungi and lichens. Monosporial culture of the fungi was obtained by the method of exhaustion on a poor potato-dextrose agar [41].

The detergent powder used was packaged household synthetic detergent of domestic Merix brand (Merima, Kruševac). Ethoxyl-oleyl-cetyl alcohol and sodium tripolyphosphate are also supplied by Merima.

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 consisted of: 3 g $NaNO_3$, 1 g K_2HPO_4, 1 g $MgSO_4$, 0.25 g $MgSO_4x7H_2O$, 0.01 g $FeSO_4x7H_2O$ and 30 g saccharose, dissolved in 1000 ml of distilled water. Detergent designated D, ethoxyl-oleyl-cetyl alcohol (AOC) and sodium tripolyphosphate (TPP) was added (1%) and the flasks incubated for 4-8 days. The flasks containing 200 cm^3 of medium were uniformly and constantly shaken on Kinetor shaker at room temperature in condition of alternate light-dark cycles [42]. The sterility of the nutrient medium was tested using mesopeptone agar.

For the determination of free organic acids 10 ml of medium was taken and mixed with 50 ml of ethanol. After incubation at 70˚C in a water bath for 1-1.5 hours, the mixture was filtered through a special filter. The filtrate then was concentrate at 50˚C - 60˚C and reduced pressure to the volume of 40 ml, transferred to a volumetric flask and made up to 100 ml after addition of a teaspoon of the active charcoal. After standing in a water bath for 30-45 min at 70˚C, 10 ml 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 [43-45].

The monosaccharides, glucose and fructose, were determined after cation-exchange chromatography on a column of AmberliteIR-120 followed by descending chromatography on Whatman No 1 paper. After reaction with suitable reagent to produce a green-blue complex, the amount of glucose and fructose were determined spectrofotometrically using a red filter in comparison with the appropriate standard curves [43].

The qualitative and quantitative content of amino acids was determined by standard method described by Sparkman [46] using aminoanalyzer BECKMAN model 120 C. Measuring conditions: stationary phase—LiChroCART 250-4, mobile phase—0.10 M acetic buffer (pH 4.4) and acetonitrile in 70:30 ratio, mobile phase rate 1.0 ml/min, fluorescent detector (Ex 263 nm, Em 313 nm).

The amount of proteins was determined by Kjeldahl method on the basis of amount of nitrogen present in fungi [47] using equation:

Amount of proteins = 6.25 x amount of nitrogen (mg)

3. RESULTS AND DISCUSION

This paper conducted a research on influence of detergent Merix (Merima, Kruševac, Serbia) and its components (ethoxyl-oleyl-cetyl alcohol and sodium tripolyphosphate) on metabolic activity of fungi *Aspergillus niger*. The metabolic changes of bioproduction of different organic compounds in various aging step of fungi in the presence of pollutant has been investigated. This fungus was isolated from the river Lepenica (Serbia) on wastewater outpouring site and was chosen because it was the most abundant there. The fungi was then grown in the 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 *Aspergillus niger* grown in such liquid nutrient medium and control medium is monitored over following biochemical parameters: amounts of free organic acids and monosaccharides on 4th and 8th day of incubation period, amount of proteins on each day of incubation period and qualitative and quantitative content of amino acids on the last day of incubation period.

Aspergillus niger did not produce carbohydrates on the 4th day of incubation period. At the end of examination period, fungi produced different amount of carbohydrates (glucose and fructose) depending on the composition of nutrient medium. Compared with the control medium, production of fructose was increased in all version of nutrient medium, except in the case when 1% of detergent was added (**Figure 1**).

Production of free organic acids was increased in all cases at the end of experimental period, but it was dramatically increased in nutrient medium with sodium tripolyphosphate added (**Figure 2**).

On the last day of experimental trial, in the control nutrient medium *Aspergillus niger* produced 15 essential amino acids while in the nutrient medium with 1% of detergent added 14 essential amino acid were produced. The detergent manifested extremely stimulating effect on bioproduction of lysine, arginine, aspartic and glutamic acid, valine, leucine, isoleucine and tyrosine. Bioproduction of other amino acids was less intensity, but still increased in comparison with those produced in control medium. Only alanine was not produced in the presence of detergent (**Figure 3**).

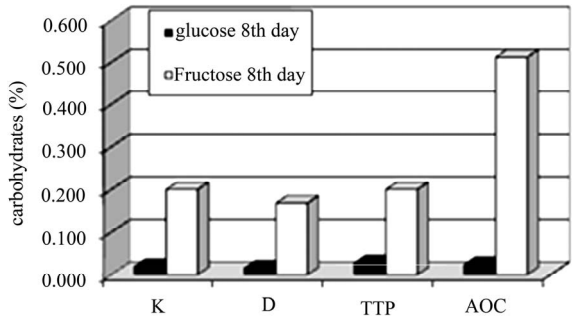

Figure 1. Amount of carbohydrates (%) produced by *A. niger* on 8th day in different liquid nutrient mediums.

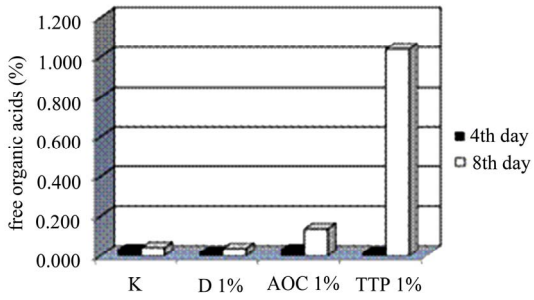

Figure 2. Amount of free organic acids (%) produced by *A. niger* on the 4th and 8th day in different liquid nutrient mediums.

Amount of proteins produced by *Aspergillus niger* in different liquid nutrient mediums was monitored on each day of 4-8 days incubating period and had differed depending on the liquid nutrient medium used and the aging step of fungi (**Figure 4**).

With the aging of fungal culture production of pro-

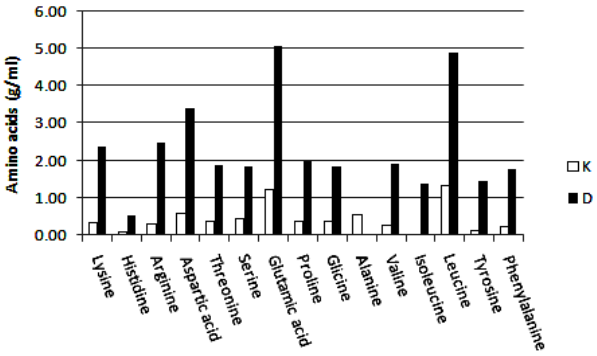

Figure 3. Qualitative and quantitative content of amino acids produced by *A. niger* on the 8th day in liquid nutrient medium with 1% detergent added.

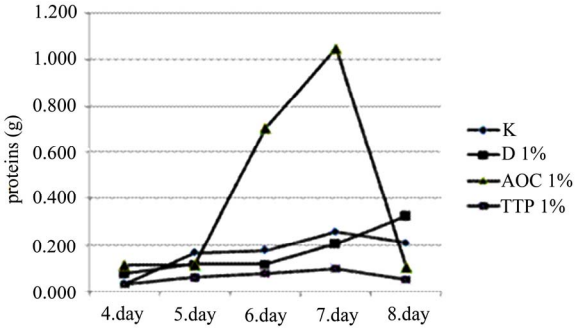

Figure 4. Amount of proteins (g) produced by *A. niger* during 4-8. day of incubating period in different liquid nutrient mediums.

teins decreased in all cases, except in the medium with 1% of detergent added in which production was a little bit higher compared with control experiment. The highest production of proteins was in the medium with 1% ethoxyl-oleyl-cetyl alcohol added on 7th day, while the lowest was in the medium with 1% sodium tripoly- phosphate added on 4th day of incubation. Sodium tripolyphosphate exhibited the highest inhibitory effect on bioproduction of proteins during the entire incubation period.

4. CONCLUSIONS

On the basis of the results obtained it can be concluded that detergent and its components added to liquid nutrient medium according to Czapek changed metabolic activity of fungi *Aspergillus niger* on a different ways, what reflected on growth and development of fungi.

The results of amino acid analyses indicated that *Aspergillus niger* is resistant to effect of detergent in concentration applied. It produced a variety of amino acids, 14 overall, and all of them were 2-10 times more abundant then in control experiment.

Bioproduction of proteins, at the end of examination period, was stimulated a little by detergent and extremely inhibited by sodium tripolyphosphate and partially by ethoxyl-oleyl-cetyl alcohol.

Detergent also exhibited high inhibitory effect on bioproduction of free organic acids while sodium tripolyphosphate and ethoxyl-oleyl-cetyl alcohol exhibited considerably stimulating effect.

Detergent powder and its components highly influence metabolic activity of *Aspergillus niger*. Thus this fungus, which is most abundant on wastewater outpouring site of river Lepenica, can metabolize detergent components for growth and biomass accumulation and play an important role in purification of river waters.

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