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BIOCHEMICAL CHANGES OF BIOPRODUCTION OF CARBON HYDRATES OF *Aspergillus niger* van Tieghem, 1867 THE INFLUENCE BY DETERGENT AND ITS COMPONENTS

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SYNOPSIS

The species of the fungi *Aspergillus niger* was isolated on the place of waste water flood. Biochemical changes of production of carbon hydrates (quantity glucose and fructose), total organic acids and total biomass from 4th to 8th day were followed on the inoculated species of fungi *Aspergillus niger* grown on liquid nutritious base according to Czapek and on a variation of the same nutritious base with detergent and the components of the same detergent: ethoxyled oleyl-cetyl alcohol and sodium tripoly-phosphate of 1% concentration. The detergent and its components (ethoxyled oleyl-cetyl alcohol and sodium tripoly-phosphate) of 1% concentration had a partial or significant influence on the bioproduction of total organic acids.

The detergent is the strongest inhibitor of the bioproduction of glucose, and particularly of fructose in relation to the control, which influences inhibition of total biomass. In the presence of the detergent of 1% concentration the total biomass is hundreds of less in relation to the control.

INTRODUCTION

Microorganisms, first of all bacteria and fungi, had the ability of biodegradation of the organic compounds, which come to the living environment by the activity of a man. Among the fungi that have this ability the filamentous fungi from the group *Deuteromycetes* or *Imperfect fungi* are especially separated, for the sake of their physiological, morphological and biochemical characteristics (Raimbault, 1981).

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

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

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

Phosphates detergents are highly caustic, they modify pH media, induce the eutrophication (the blooming of algae) in fresh waters, the toxin release and the quantity decrease of dissolved oxygen (Tunney et al., 2006; McGrath & Quinn, 2004).

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

The linear alkyl-benzene sulphonated (LAS) is the anion surface active agent used almost only in detergents more than 30 years. LAS is very easily degraded under the activity of microorganisms in the waste water plants (Waters & Feijtel, 1995; Holt et al., 2003). The primary LAS biodegradation on the activated sludge is higher than 99% (EU COMMISSION, 1997). The traces of LAS in natural waters (Itrich & Federle, 1995) and in the soil (Küchler & Schnaak, 1997) continue the quick biodegradation (half life of LAS is about 0,15-0,5 days). The ultimate biodegradation of LAS quantity is 95-98 % so that they are not harmful for the environment and living creatures.

By the analysis of the activated sludge microbiological community in waste water plants and waste waters passing through the populated places the great number of different fungi species degrading detergents as in aerobic so in anaerobic conditions as well were identified (Sanz et al., 2003; Bonin et al., 2004).

SUBJECT AND METHOD OF RESEARCH

A monosporial culture of the fungi species *Aspergillus niger* van Tieghem isolated and determined from the river basin of Lepenica (the place of waste water flood, sewage). When deciding which species of fungi to use, the quantitative representation with the fungi species in precisely determined capacity of the water specimen was taken into consideration. The isolated and determined fungi species were maintained in a chamber with a constant temperature of 4°C (± 0,5°C), on potato-dextrose agar slant, in sterile conditions.

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

During the experiment, the fungi were grown on the sterile nutrient base according to Czapek (g/L), of the following composition: $NaNO_3$ -3, K_2HPO_4 -1, $MgSO_4$ –1, $MgSO_4x7H_2O$ - 0,25, $FeSO_4x7H_2O$ - 0,01, saccharose-30, distilled water-1000 ml (control-K) and on variant of liquid nutritious base, according to Czapek, with detergent MERIX, (”Merima”, Kruševac, mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 1%. The variant of the liquid nutritious base was stored in Erlenmeyer bottles (200 ml of base in 250 ml bottles). Erlenmeyer bottles were placed on an electric shaker, thus enabling uniform and constant mixing (aeration of the fungi). The experiments were carried out at room temperature, under alternate light and dark (Stojanović, 1990).

Following analysis of the fungi *Aspergillus niger* grown on a liquid nutritious base, according to Czapek (control) and on the variant of liquid nutritious base, according to Czapek, with detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in concentration of 1%, were observed: production of carbon hydrates (quantity glucose and fructose), total organic acids and total biomass from 4^{th} to 8^{th} day.

Total organic acids of the specimen are isolated by the ion-changeable columns (cation, Aberlite-R-120 type), their quantity are determined according the same procedure as for the free organic acids (Veličković, 1971; Stojanović et al., 2001, 2002).

The separation of carbon hydrates (monosaccharides) of used species of fungi grown in all variants of bases was achieved with the ion-changeable columns and with the use of chromatographic methods (the descending chromatography). The quantity of glucose and fructose was measured by the spectrophotometric methods based on the use of the standard curve for investigated sugars and suitable reagents that transform monosaccharides into colored state (the blue-green complex) (Veličković, 1971).

The quantity of biomass of the fungi species *Aspergillus niger* was determined on the basis of the mass difference between dry filter paper and the total mass with

mycelium of the fungus. The quantity of biomass is expressed in grams (g) (Stojanović et al., 1986).

RESULTS AND DISCUSSION

The production of total organic acids of the fungi species *Aspergillus niger* grown on the all mentioned variants of nutritious bases on the 8th day (with aging of the culture) was considerably or significantly higher in relation to the 4th day, for example it was more than 50 times in the variant of nutritious base with sodium tripoly-phosphate in concentration of 1%, as presented in Figure 1.

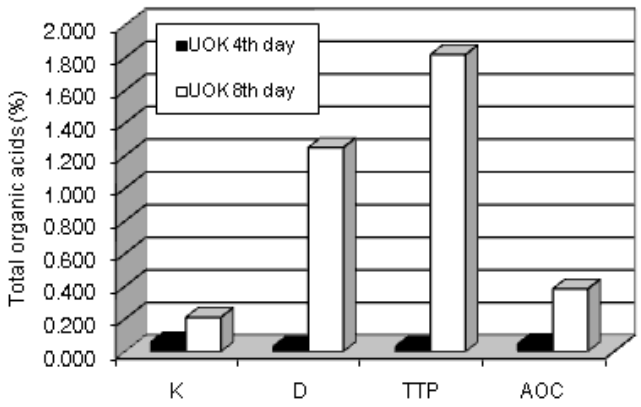

Figure 1: The quantity of total (mark UOK) organic acids (in %) of Aspergillus niger grown on the nutritious base according to Czapek (control-K) and the variant of nutritious base with detegent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 1% on the 4th and the 8th day.

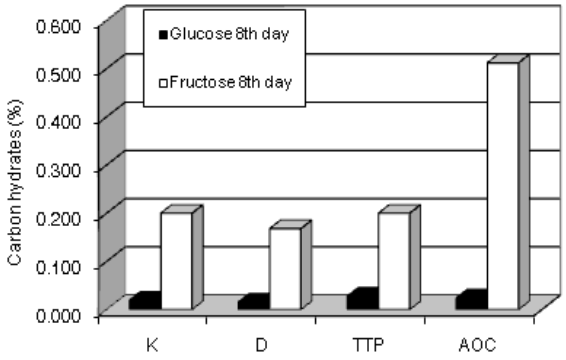

Figure 2: The quantity of carbon hydrates (in %) of *Aspergillus niger* grown on the nutritious base according to Czapek (control-K) and the variant of nutritious base with detegent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 1% on the 8th day.

The greatest production of total organic acids the fungus achieved on the media with added sodium tripoly-phosphate and detergent in concentration of 1%. Values were considerably higher in relation to control on the 8th day.

The detergent and sodium tripoly- phosphate had a similar inhibitory effect in the early phase of the fungi growth but strong stimulating effect in the last phase, especially sodium tripoly phosphate. Species of fungi *Aspergillus niger* grown on the liquid nutritious base (according to Czapek) and on the variant of nutritious base with detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in concentration of 1% produced carbon hydrates only 8th day, as presented in Figure 2.

In relation to the control nutritious base the production of glucose of the fungus *Aspergillus niger* was significantly less on the nutritious base with added detergent of slightly less on the nutritious base with added ethoxyled oleyl-cetyl alcohol in concentration of 1%.

The production of fructose of the same fungi species was double less on the media with added ethoxyled oleyl-cetyl alcohol and weakness on the media with added detergent in concentration of 1%. The detergent is the strongest inhibitor of the bioproduction of glucose, and particularly of fructose in relation to the control.

The total biomass of the fungus *Aspergillus niger* grown on the liquid nutritious base, according to Czapek (control) and on the variant of nutritious base with detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in concentration of 1% was variable depending on the nutritious base type, as presented in Figure 3.

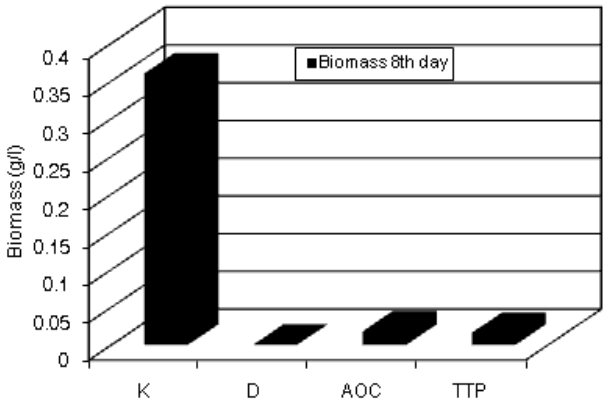

Figure 3: The quantity of biomass (g/l) of *Aspergillus niger* grown on the nutritious base according to Czapek (control-K) and the variant of nutritious base with detegent (mark-D), sodium tripoly-phosphate (mark-TTP) and ethoxyled oleyl-cetyl alcohol (mark-AOC) in concentration of 1% on the 8th day.

The quantity of the total biomass of the fungus *Aspergillus niger* grown on the control nutritious base was significantly higher in relation to the biomass of the same

fungus grown on the variant of nutritious base with detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol in concentration of 1% vol.

Detergent of 1% concentration showed the most significant inhibitory effect on the total biomass bioproduction in relation to the control.

CONCLUSION

On the bases of the obtained results it can be concluded that detergent, sodium tripoly- phosphate and ethoxyled oleyl-cetyl alcohol added in the liquid nutritious base in concentration of 1% have the ability to change the production of carbon hydrates (quantity glucose and fructose), total organic acids and total biomass from 4th to 8th day were followed on the inoculated species of fungi *Aspergillus niger.*

The detergent and sodium tripoly-phosphate had a similar inhibitory effect in the early phase of the fungi growth but strong stimulating effect in the last phase, especially sodium tripoly-phosphate.

The detergent is the strongest inhibitor of the bioproduction of glucose, and particularly of fructose in relation to the control, which influences inhibition of total biomass. In the presence of the detergent of 1% concentration the total biomass is hundreds of less in relation to the control.

REFERENCES:

BONIN, P., CRAVO-LAUREAU, C., MICHOTEY, V. & HIRSCHLER-REA, A. 2004: The anaerobic hydrocarbon biodegrading bacteria: an overview. – *Ophelia*, 58: 243–254.

EU COMMISSION, *DG III*, 1997: Study on the possible problems for the aquatic environment related to surfactants in detergents. - WRC, EC 4294.

HOLT, M.S., FOX, K.K., DANIEL, M. & BUCLAND, H. 2003: LAS and Boron monitoring in four catchments in the UK contribution to GREAT-ER. - *The Science of the Total Environment*, 314-316: 271-288.

ITRICH, N.R. & FEDERLE, T.W. 1995: Primary and ultimate biodegradation of anionic surfactants under realistic discharge conditions in river water. - *SETAC Meeting*, Vancouver, Canada.

KÜCHLER, T. & SCHNAAK, W. 1997: Behavior of LAS in sandy soils with low amounts of organic matter. – *Chemosphere*, 35: 153-167.

MORENO, A., FERRER, J. & BERNA, J.L. 1990: Biodegradability of LAS in a Sewer System. - *Tenside Surfactants Detergents*, 27: 312-315.

MCGRATH, J.W. & QUINN, J.P. 2004: Biological Phosphorus Removal. - In: Valsami-Jones, E. (Ed.), Phosphorus in Environmental Technology - *IWA Publishing*, pp. 272-286.

RAIMBAULT, M. 1981: "Fermentation en milieu solide: croissance de champignons filamenteux sur substrats amylacés". - *ORSTOM-Paris*; Série Travaux et Documents, 127: 291 pp..

RAIMBAULT, M. 1998: General and microbiological aspects of solid substrate fermentation, *Electronic Journal of Biotechnology,* 1(3), Issue of Decembar 15, 1998, Available from: http://www.scielo.cl/scielo.php?pid=S0717-34581980003000007&script=sci_arttext.

SANZ, J.L., CULUBRET, E., DE FERRER, J. MORENO, A. & BERNA, J.L. 2003: Anaerobic biodegradation of linear alkyl benzene sulfonate (LAS) in upflow anaerobic sludge blanket (UASB) reactors. - Centre of Molecular Biology, Autonoma University of Madrid, Unit of *Applied Microbiology*, 14(1): 57-64.

SAUCEDO-CASTAÑEDA, G., LONSANE, B.K., NAVARRO, J.M., ROUSSOUS, S. & RAIMBAULT, M. 1992a: Potential of using a simple fermenter for biomass built up, starch hydrolysis and ethanol production: Solid state fermentation system involving *Schwanniomyces castellii*. - *Applied Biochemistry and Biotechnology*, 36: 47-61.

SAUCEDO-CASTAÑEDA, G., LONSANE, B.K., NAVARRO, J.M., ROUSSOUS, S. & RAIMBAULT, M. 1992b: Maintenance of heat and water balances as a scale-up criterion for the production of ethanol by *Schwanniomyces castellii* in a solid state fermentation system. - *Process Biochemistry*, 27: 97-107.

STOJANOVIĆ, J., RISTANOVIĆ, J. & VUČETIĆ, J. 1986: A contribution to understanding of biochemical properties of some fungi i n v i t r o (in Serbian). – *Mikrobiologija,* Belgrade, 23 (1): 73-79.

STOJANOVIĆ, J. 1990: Uticaj deterdženata na biohemijska svojstva nekih gljivica *i n v i t r o*. - Doktorska disertacija, Univerzitet u Kragujevcu.

STOJANOVIĆ, J., STOJANOVIĆ, M. & MILOVANOVIĆ, A. 2001: Influence of detergent and some components of detergents on bioproduction of organic acids and enzymatic activity of fungi. - *Acta Veterinaria*, Beograd, 51 (2-3): 171-176.

STOJANOVIĆ, J., VELIČKOVIĆ, D. & VUČETIĆ, J. 2002: Influence of detergent and some components of detergents on bioproduction of organic matter and enzymatic activity of two fungal Species. - *Acta Veterinaria*, Beograd, 52 (4): 267-272.

TUNNEY, M., MORIARTY, T., MULLAN, A., MCGRATH, J.W., QUIN, J.P. & ELBORN, J. 2006: Effect of reduced pH on inorganic polyphosphate accumulation by *Burkholderia cepacia* complex isolates. - *Letters in Applied Microbiology*, 42: 617 - 623.

VELIČKOVIĆ, D. 1971: Contribution to study of aminoacidic composition dynamics of protein complex and aminoacids in apple fruits during the vegetative period and storage. - PhD Thesis, University of Belgrade.

WATERS, J. & FEIJTEL, T.C.J. 1995: AISE/CESIO environmental surfactant monitoring programme: outcome of five national pilot studies on LAS. – *Chemosphere*, 30: 1939-1956.

BIOHEMIJSKE PROMENE BIOPRODUKCIJE UGLJENIH HIDRATA GLJIVE *ASPERGILLUS NIGER* USLOVLJENE DETERDŽENTOM I NJEGOVIM KOMPONENTAMA

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SAŽETAK

U našem radu prikazani su rezultati uticaja deterdženta „MERIX“ (merima, Kruševac) i kvantitativno najzastupljenijih komponenti deterdženta: natrijum tripolifosfata i etoksilovanog oleil-cetil alkohola 1% koncentracije. Pratili smo: produkciju ugljenih hidrata (glukoze i fruktoze), ukupne organske kiseline i ukupnu biomasu *Aspergillus niger* van Tieghem.

Rezultati istraživanja su pokazali da je deterdžent uticao na povećanje pH sredine i značajno smanjenje redoks potencijala u odnosu na kontrolu. Proteolitička aktivnost u podlozi sa deterdžentom bila je veomna intenzivna 6. dana ogleda, ali potpuno inhibirana 7. i 8. dana ogleda, što se ispoljilo na inhibiciju ukupne biomase gljive.

Deterdžent i natrijum tripolifosfat su inhibirali produkciju ukupnih organskih kiselina u početnim fazama rasta i razvoja gljive, ali su delovali veoma stimulativno u kasnijim fazama, naročito natrijum tripolifosfat.

Deterdžent je najviše inhibirao bioprodukciju glukoze i delimično fruktoze u odnosu na kontrolu, što se odrazilo na inhibiciju ukupne biomase.

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