INFLUENCE OF FINE-GRAINED MONTMORILLONITE ON MICROFUNGAL PELLETS GROWTH IN AQUEOUS SUSPENSIONS

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Abstract: The paper presents an inhibition effect of clay mineral – montmorillonite – on the growth of microscopic filamentous fungus *Aspergillus niger* in the aqueous solution. The significant reduction in growth of the final size of spherical fungal pellets as well as total amount of produced microbial biomass was found out. Within the observed range of additions of clay mineral of 1, 5, 10, 15 and 20 g in the total volume of the 80 ml suspension, this size was in indirect relation to the weight of montmorillonite. However, the most significant inhibition effect was observed at the lowest concentration of the sorbent (1 g). Microscopic analysis of pellets referred to the presence of mineral particles in their pore structure and the distribution of particles in the spatial structure of fungal hyphae was variable. The experiment clearly demonstrated an inhibition effect of montmorillonite. This inhibition could be answered by the experiments focused on the detection of the influence of size and shape of inorganic sorption particles together with the influence of the physicochemical properties of its surface. It could be stated that the simultaneous application of the microscopic fungus *Aspergillus niger* and the clay mineral montmorillonite for decontamination of waste waters should be disadvantage due to their interaction if compared with the decontamination based on bioaccumulation and sorption separately.

Key words: montmorillonite, *Aspergillus niger*, microfungal pellets, radial growth

1. Introduction

The *Aspergillus niger* species is an ubiquitous soil microscopic filamentous fungus (ŠIMONOVIČOVÁ, 2013). It could be used in bioaccumulation of various potentially toxic elements and in bioremediation of contaminated sites (ENONTIEMONRIA *et al.*, 2012; SOLEIMANIFAR *et al.*, 2011; ŠIMONOVIČOVÁ *et al.*, 2010; ŽEMBERYOVÁ *et al.*, 2009; WASAY *et al.*, 2010).

The simultaneous occurrence of microscopic filamentous fungi and microcrystalline inorganic silicates is often in natural systems. Both components frequently occur in soils and sediments and they are able to decrease the concentration of various water-soluble substances in their surrounding environments. For the transport of these substances from the surrounding environment, the presence of water in its fluid phase is required, which significantly increases surface for interaction of the sorbent, also due to decreasing of particle size to smaller particles and also it

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allows to implement significantly more effective sorption mechanisms (for example cation exchange).

Bioaccumulation properties of microscopic fungi and sorption properties of microcrystalline silicates result in consideration about their potential application for decontamination of the environment (FOMINA and GADD, 2002; SARASWATHY and HALLBERG, 2005). Thereby bioaccumulation properties of microscopic fungi as well as sorption properties of microcrystalline silicates are known, overall decontamination effect in technological processes followed by implementation of bioaccumulation and sorption units may be relatively well estimated. On the contrary, decontamination effect of the simultaneous application of microscopic fungi and silicate sorbents cannot be estimated because it is complicated by their interactions. Therefore, it is necessary to estimate it experimentally. It can be supposed that this effect will be lower than sum of effects of both components. The main reason should be an inhibition of transport of the solution towards the sorbent surface due to the decreasing of the circulation of fluid phase in the surroundings of fungal pellets as well as directly inside the pellets. Another reason could be an inhibition effect of the sorbent on the growth of microscopic filamentous fungi.

The aim of the paper is to carry out an assay of the influence of the clay mineral montmorillonite on the growth of microscopic filamentous fungus *Aspergillus niger* in the aqueous environment. The selection of montmorillonite for this study is because of this mineral belonging to very frequent component of various soils. It also represents inexpensive industrial products with adequate purity. The overall strategy of performed experiments was based on the model system that included microscopic filamentous fungus, standard culture medium and clay mineral with no impurities identified by X-ray diffraction method and it was also enough characterized by its particle size in aqueous suspension.

2. Material and methods

The influence of montmorillonit on the growth of microscopic filamentous fungus *Aspergillus niger* was observed in 70 ml Sabouraud medium composed of glucose, peptone and distilled water, with addition of 10 ml of aqueous suspension of fungal spores. The *A. niger* strain was originally isolated from the Šobov locality (ŠIMONOVIČOVÁ *et al*., 2013). The monomineral sample of montmorillonite in addition of 1, 5, 10, 15 and 20 g was dispersed in the suspension of the culture medium with fungal spores. The cultivation was carried out for 7 days at 25 °C in 100 ml conic flasks. The flasks were then shaken using Unimax 2010 shaker (Heidolph, Germany) at 130 rpm. There were three replicated runs for each experiment. The fungal pellets were washed by 500 ml of distilled water at the end of the experiment.

Montmorillonite used in the experiment was obtained by sedimentation of 4 % aqueous suspension of bentonite from the Stará Kremnička – Jelšový Potok site. The sedimentation conditions were set up to acquire the particle size of lower than 5 μm as calculated according to Stokes' equation. The distribution of size of montmorillonite particles was then determined by the particle size laser analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK). This analyzer is applicable for the analysis of particle size in the range from 0.02 to 2 000 μm.

The size of fungal pellets was determined according to the photographs of the thin layer of suspension in the Petri dish, in which pellets were in mono layer with negligible overlapping of the particles. The microstructure of pellets was observed by optical microscope (Jenalumar, Carl Zeiss Jena, Germany) and photographs were recorded by digital camera (Camedia C-5060, Olympus, Japan). To increase background contrast, pellets were colored by commercial histological pigment based on methylene blue (Lactophenol Cotton Blue, HiMedia Laboratories Pvt. Ltd., India).

3. Results and discussion

The distribution of montmorillonite particle size, determined using particle size laser analyzer (Fig. 1) shows that main content of the clay mineral is formed by particles lower than 10 μm. In regard of the mineralogical homogeneity of the montmorillonite samples, these particles also formed the main weight ratio of the sample. The result of the analysis shows significant difference against diameter of particles derived from the Stokes' equation. The content of non-clay impurities in the bentonite fractions obtained from the Stará Kremnička – Jelšový Potok site with the particle size lower than 10 μm is negligible (JESENÁK, 2007).

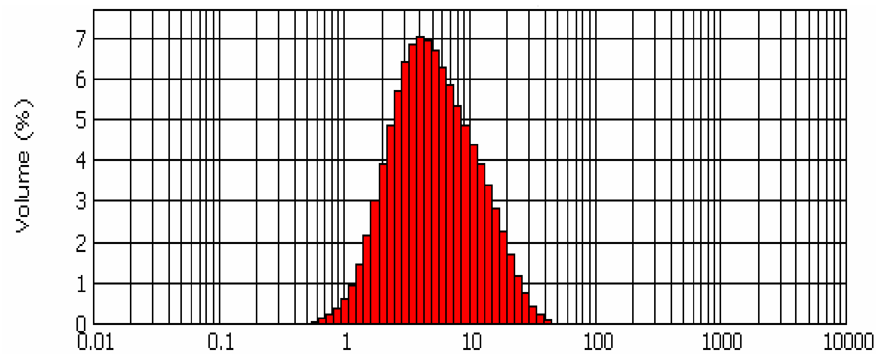

Fig. 1 The distribution of the particle size of montmorillonite isolated from bentonite obtained from the Stará Kremnička – Jelšový Potok site using a particle size laser analyzer Mastersizer 2000 (Malvern Instruments Ltd., UK). (The particle size is expressed in micrometers.)

The selection from the series of experiments focused on the determination of the influence of montmorillonite on the fungal pellet growth is shown in Fig. 2.

It can be seen that sizes of the microfungal pellets formed in the presence of dispersed particles of montmorillonite are markedly lower than sizes of pellets formed in culture medium without addition of montmorillonite (Fig. 3). The size of the first type of pellets ranges from 2 to 6 mm with the highest amount in the range of 3-4 mm. Pellets formed in the presence of different additions of montmorillonite have their size lower than 1 mm with the highest amount in the size of 0.5 mm. The experiments show that approximately 1 wt% of clay mineral in culture medium markedly reduces the pellet size (almost 10-fold), however, another increasing of montmorillonite has only minimal influence on the pellet sizes. Another result of the experiments is the fact that with increasing weight of clay mineral, relative amount of produced fungal biomass is decreasing. This statement could be derived from the surface of pellets on the Petri dishes (Fig. 2).

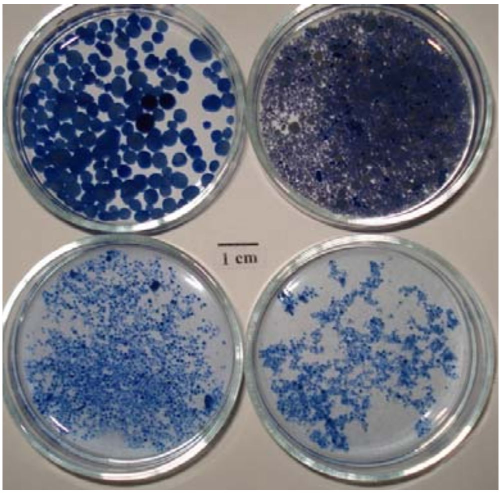

Fig. 2. Microfungal pellets of the *A. niger* species formed after 7-day cultivation at 25 °C in 80 ml of Sabouraud culture medium (upper left corner). All other Petri dishes show fungal pellets of the same fungal strain formed in the presence of different amounts of the clay mineral montmorillonite under the same cultivation conditions (1 g – upper right corner, 5 g – lower left corner, 10 g – lower right corner). Fungal pellets were colored with commercial histological pigment based on methylene blue (Lactophenol Cotton Blue, HiMedia Laboratories Pvt. Ltd., India).

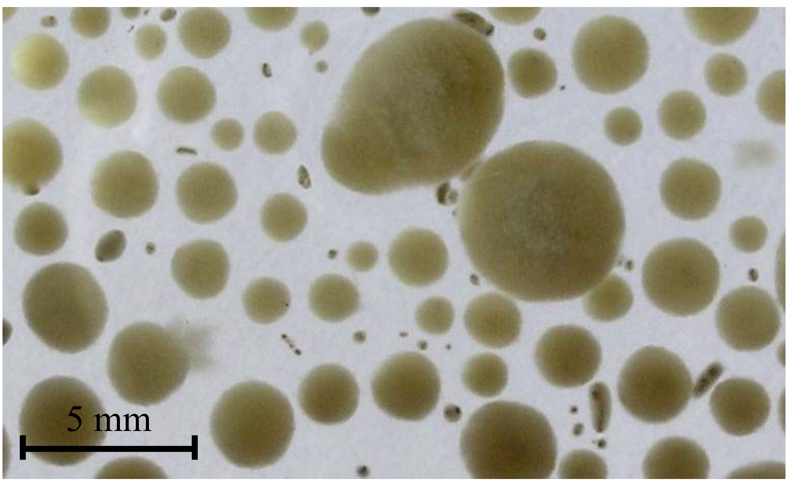

Fig. 3. Variability in shape and size of the microfungal *A. niger* pellets produced in the presence of the clay mineral montmorillonite. The size of the largest pellets is approx. 5 mm.

Morphology and size of pellets under variable conditions as volume of fungal inoculum and medium, carbon and nitrogen source, initial pH and temperature or adding of mineral microparticles was studied by many other authors (FENG *et al*., 2003; GONCIARZ and BIZUKOJC 2014; LÓPEZ *et al*., 2004; PETRE *et al*., 2005; KIM and SONG, 2009). Reduction of pellet size after addition of bentonite and kaolinite as well as after addition of talc microparticles to *Aspergillus glaucus* inoculum was observed by FOMINA and GADD (2002) and GONCIARZ and BIZUKOJC (2014), respectively. According to KIM and SONG (2009) the agitation speed is the most important control factor. At the lower agitation speed of 100 rpm the large pellets are formed and the enzymatic activity is higher than that of small pellets culture at 150 rpm. A new strategy for shaping fungal morphology by modifying polarized growth genes applied in submerged fermentation in bioreactor which could be valuable for morphology improvement of industrial filamentous fungi is documented by CAI *et al*. (2014).

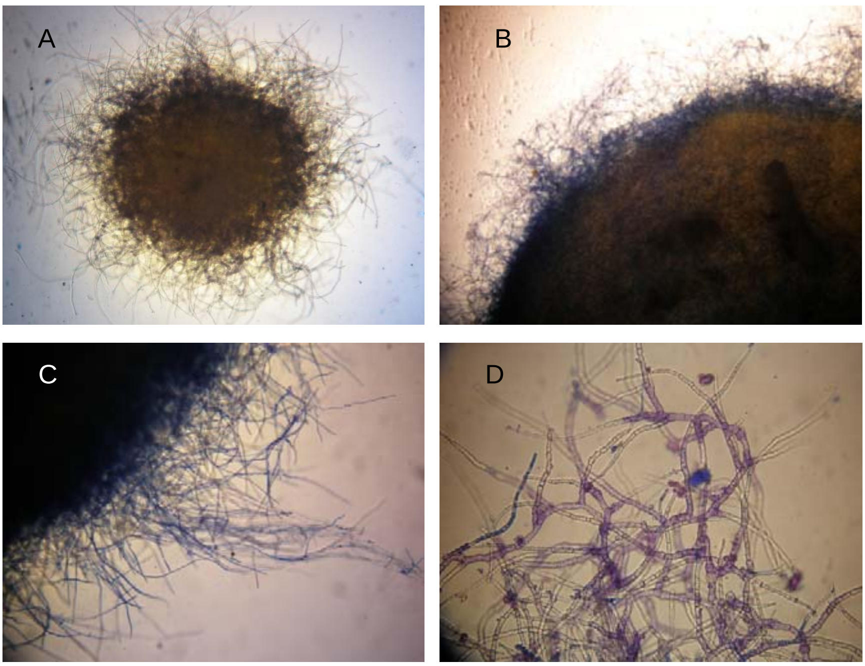

Fig. 4. The structure of single pellet of the *A. niger* fungus containing closed particles of montmorillonite (mag. 40×) (A). Particles of montmorillonite (dark objects) inside the filamentous structure of the fungal pellet (mag. 78×) (B). The detail of the pellet surface (mag. 157×) (C). The detail of fungal hyphae at the pellet surface (mag. 252×) (D). (The size of the pellet is 2 mm.)

Fig. 4 shows several details of spherical fungal pellets formed in the culture medium with addition of clay mineral montmorillonite. It could be seen that clay particles are fixed within the filamentous structure of microscopic fungus (Figs. 4A, B). However clay particles are not fixed on the pellet surface regularly. The amount of clay particles decreases from the middle of the pellet to its surface. This change in frequency of the occurrence of montmorillonite particles could be well explained by decreasing in the density of hyphal net towards to the pellet surface (Figs. 4C, D). However, the amount of clay mineral particles in individual volume parts of pellets is not continuous. It depends on their distance from their geometric center. Considerably diffusion surface of pellets shows that the objectification of dimensional properties of pellets, for example by image analysis, is very limited by considerably subjective selection of abstract line, which serves for separation of the pellets from its surroundings.

These findings could be viewed as follows. The first view is the application of microscopic filamentous fungi and clay minerals in decontamination of the environment. It seems that such application of microscopic fungi and silicate sorbents would not offer any significant advantages in decontamination technologies. However, the overall influence of the interaction of both components on the decontamination effect could be shown only by the experimental observation. The second view on the findings is connected to reasons of the inhibition influence of clay minerals on the growth of microscopic filamentous fungi. The explanation of these reasons could be shown by experiments focused on intensity of this influence.

4. Conclusion

The main result of the experiments was a clear confirmation of the significant reduction of the size of spherical pellets of the *Aspergillus niger* microscopic fungus due to the inhibition of its growth by clay mineral montmorillonite. There could be various reasons of this effect such as spherical inhibition of the growth, changes resulting from physicochemical interactions of the clay mineral surface with the surface of microscopic fungi as well as from interactions of clay with the components of culture medium. One of the problems of the research focused on the explanation of the effects is that none of the listed properties could be changed independently when fine-grained particulates are used.

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