Study of the degradation patterns of thermophilic fungi from special digested wastewater sludge samples

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Abstract: Digested wastewater sludges have high content of thermophilic fungi because of the anaerobe biodegradation. This study shows the ecophysiological properties (cellulose, hemi-cellulose, lignin and fatty-acid ester degradations) of thermophilic fungi which were isolated from digested wastewater sludge samples. The samples came from three Hungarian wastewater treatment systems. In this study four types of mycological agars were applied. This study shows digestion properties and growing temperature range of eight thermophilic fungal species. This study demonstrated wide ranges of digestion properties. Most of the isolated species are appropriate for degradations of several biopolymers.

Keywords: fungal species, wastewater treatment, ecophysiological properties

Introduction

The biodiversity and the ecosystems are basic for the human and nature. An important part of the eosystems are the fungi (psychrophilic, mesophilic and thermophilic species). About 100.000 fungal species have been described worldwide *(Valencia et al., 2013)*. Fungi have been used in pharmaceutical and biotechnological industries to produce important materials *(Lange et al, 2012)*. Thermophilic fungi also have been used in biogas production. Wastes from food industry and other agricultural systems have high methane potential and high content of thermophilic fungi *(Palatsi et al., 2009)*. In biogas production the lipid content is hydrolysed by extracellular enzymes of fungi. Fatty acids are degraded through ß-oxidation process *(Palatsi et al., 2009)*.

The average growing temperature range of the mesophilic and thermophilic fungi are between 10^{o}C and 60^{o}C. While, the optimal growing temperature of extreme thermophilic fungi is up to 90^{o}C *(Sterflinger et al., 2012)*. Most of the thermophilic fungal species can use carbon and nitrogen contents of materials to their processes *(Souza et al., 2013)*. The best nitrogen sources are potassium-nitrate, sodium-nitrate and asparagine *(Wagner et al., 2013)*, while the ammonium-nitrate and sodium-nitrite are poor sources to thermophilic fungi *(Chmielewski et al., 2012)*. The high rate of unsaturated fatty acids is the reason of the resistant cell membrane in thermophilic fungal species *(Palatsi et al., 2009; Watanabe et al., 2012)*. Therefore, the transport processes could work at higher temperature through the cell membrane *(Dobolyi et al., 2008; Souza et al., 2013)*. In the study of *Wagner et al. (2013)* nine complex organic substrates from three biochemical classes (protein-, lipid-, and cellulose-rich) were investigated in batch experiments and compared with controls in order to evaluate their potential use as substrates for biogas production and in order to have a comparable set of data, which independent of digestion system, reactor type, and design. The complex substrates were applied with a constant final carbon concentration to facilitate comparability *(Wagner et al., 2013)*.

The study of *Watanabe et al. (2012)* described the production system of biofuels by cellulose degradation capacity of fungi. The cellulose degradation is one of the most important parts of the biofuel production. The work reported the most 25 powerful cellulose-degrading fungi. The study of *Souza et al. (2013)* presented the enzymatical background of cellulose degradation. The enzymatic degradation of cellulose involves a complex set of enzymes as cellulases: three most relevant classes are endoglucanases,

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exoglucanases and ß-glucosidases *(Souza et al., 2013)*. Endo and exoglucanases hydrolyze internal ß-1,4 linkages of cellulosic polymers to cellooligosaccharides and cellobiose and that will be hydrolyzed by ß-glucosidases to glucose *(Watanabe et al., 2012; Souza et al., 2013)*. The process needs high cellulose concentration.

Several thermophilic fungi have been determined in a digested wastewater sludge. The fungal contamination depended on the origin of sludge: coming from communal or industrial wastewater *(Sterflinger et al., 2011)*. The complex, cooperative and dynamic processes of digestion involved the microbiological communities and a rapid community structure succession. Some species of the microbiological communities could degrade many special materials (e.g. cellulose, hemicellulose, lipids, fatty-acids). Studying the changes of the microbe communities was important to follow the material transporting processes *(Zhao et al., 2013)*. The possible transport processes should determines the species composition of the microbiome.

Materials and methods

Three types of digested wateswater sludge samples were used in our research. These samples came from three different hungarian wastewater treatment systems. Three types of agars were used to determine the thermophilic fungal species from the samples.

Digasted wastewater sludge samples

Three types of wastewater sludge samples were used in this research which came from three different hungarian wastewater treatment systems. These samples came from the wastewater treatment systems of Bánhalma, Hungary (Sample 1^{st}), Pálhalma, Hungary (Sample 2^{nd}) and Kecskemét, Hungary (Sample 3^{rd}).

The mycological agars

Four types of mycological agars were used in the experiment. The agars were the Potatoe-glucose agar, Malate-extract agar, Martin-type agar and the Mycrocristalle cellulose agar. These agars had some unique content which were fit to growing fungus. The Potatoe-glucose agar content potatoe-extract and glucose in high concentrations. In the Malate-extract agar were malate-extract and élesztő-extract. In the Martin-type agar were sacharose and K_2HPO_4. In the Mycrocristalle cellulose agar were KNO_3 and élesztő-extract and peptone in high concentrations.

The thermophilic fungus species were determined from the wastewater sludge samples quantitatively . All of the agars were used to all of the samples in the experiment. So the optimal growing temperature and the optimal degradation properties could be determined because on each agars could growen only the fungus species which could use the agar contents (e.g. cellulose, peptone).

All of the sample with all of the fungus species were temperatured in a heated box with temperature settings (the determinded temperature was 65°C, 68°C and 71°C).

Results and discussions

The ammount and the quality of thermophilic fungus were measured in the wastewater sludge samples. High numbers of thermophilic fungus species were determined in the wastewater sludge samples. We found eight species in the samples but these species were not in all of the samples because the species were growed only on the agars which contents the best materials to them.

Ammount of thermophilic fungus in samples

First of all the ammount of thremophilic fungus were measured in the wastewater sludge samples (Figure 1.).

Sample 1^{st} (Bánhalma, Hungary) – $3,7 \times 10^4$ CFU/g fungus ammount

Sample 2^{nd} (Pálhalma, Hungary) – $4,8 \times 10^3$ CFU/g fungus ammount

Sample 3^{rd} (Kecskemét, Hungary) – $7,7 \times 10^4$ CFU/g fungus ammount

Determiations of isolated fungus species

Eight thermophilic fungus species were determined in the watewater sludge samples.

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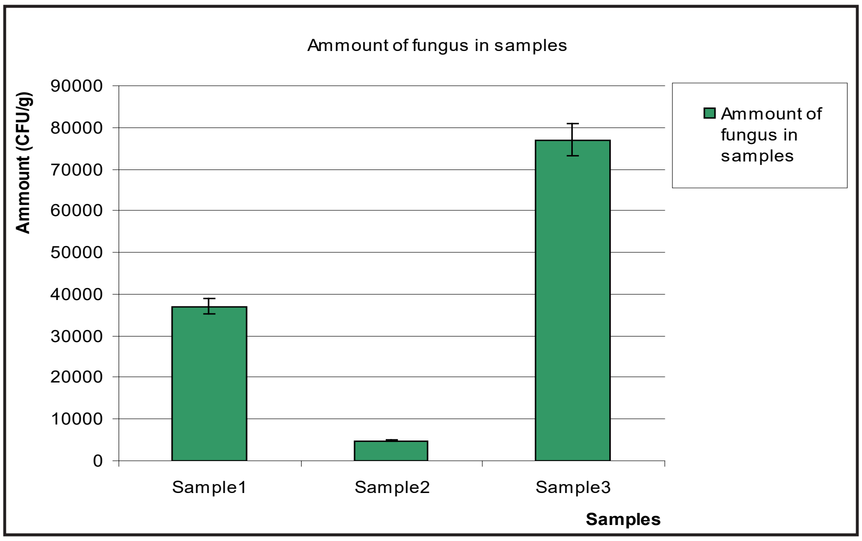

Figure 1. The ammount of thermophilic fungus in samples

The following species were found: *Malbranchea cinnamomea*, *Thermomyces lanuginosus*, *Scytalidium thermophilum*, *Myceliophthora thermophila*, *Paecilomyces sp.*, *Thermoascus aurantiacus*, *Myriococcum thermophilum* and *Rhizomucor pusillus*. An international standard were used to determined which isolated fungus were which fungus species.

The degradeted wastewater sludge is a unique matter because it has some organic matters and high water content. The thermophilic fungus could growing if the environment contains high water ammount and several organic matters (e.g. cellulose, lignin, hemi-celluloses). That was the reason of the research of organic matter degradation properties.

Degradation properties of isolated fungus

The following table (Table 1.) shows the degradation properties of each isolated thermophilic fungus species.

In comparision of fungus species cellulose degradated by three species, xilan, mannan and fatty-acid ester by seven species, lignin and ceratine by only one fungi, and phospathe by four species.

In an other comparision (in the point of isolated species): the most important organic matter cellulose could degradated by *Thermomyces lanuginosus*, *Scytalidium thermophilum*, and *Paecilomyces sp*. Three matters from seven organic matters could degredated by seven fungus. The mannan, xilan were not degradeted

Table 1. The degradation properties of isolated fungus species

Isolated species	Degradation properties (+ or -)						
	cellulose	xilan	mannan	lignin	fatty-acid ester	ceratine	phosphate
Malbranchea cinnamomea	-	+	+	-	+	-	-
Thermomyces lanuginosus	+	+	+	-	+	+	+
Scytalidium thermophilum	+	+	+	-	+	-	+
Myceliophthora thermophila	-	+	+	-	+	-	+
Paecilomyces sp.	+	+	+	+	+	-	+
Thermoascus aurantiacus	-	+	+	-	-	-	-
Myriococcum thermophilum	-	+	+	-	+	-	-
Rhizomucor pusillus	-	-	-	-	+	-	-

by only one fungi *Rhizomucor pusillus*. This fungi could degrade only the fatty-acid ester. Most of the fungus could degrade five or six matters from seven organic matters. This could be a reason of fungus methabolic proccesses.

The thermophilic fungus could use the same matters than the other fungus but they use these on higher temperature. This is the reason we measured the optimal growing (degrading) temperature. There were no significant difference between the optimal growing temperatures of isolated fungus. The growed between 65°C and 71°C. Most of the fungus were growed at 65-68°C. Only *Rhizomucor pusillus* growed at 71°C. The optimal growing temperaure means the optimal size of fungus in three days old life.

Conclusions

The digested wastewater sludge contains several fungus species (and wilde range of thermophilic fungus species). The sludge contains several organic matters which could use by thermophilic fungus to their methabolical processes. The eight isolated thermophilic fungus species were in all samples and this is the reason of the wild range of degrading. The cellulose and the other matters are useful to fungus growing. The thermophilic fungus could use these matters but they must degrade to use these in better chemical formats. We recommend to research the other important matters (e.g. organic matters in more difficult chemical formats) degradation because we would like to determine the whole degradation spectrum of thermophilic fungus.

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