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Isolates identification and characteristics of microorganisms in biotrickling filter and biofilter system treating H_2S and NH_3

YU Guang-hui[1,2], XU Xiao-jun[2], HE Pin-jing[1,*]

1. State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Shanghai 200092, China. E-mail: solidwaste@mail.tongji.edu.cn
2. School of Environmental and Civil Engineering, Qingdao Tech University, Qingdao 266033, China

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Abstract

A combination system of biotrickling filter (BTF) and biofilter (BF), adopting surfactant-modified clinoptilolite and surfactant-modified wood chip as the media respectively, was applied to treat H_2S and NH_3 simultaneously. The identification and sole carbon sources utilization patterns of isolates in the combination system were studied by Biolog system. The isolates were identified as *Bacillus sphaericus*, *Geobacillus themoglucosidasius* (55°C) and *Micrococcus luteus* (ATCC 9341) in BTF, and *Aspergillus sydowii* (Bainier & Sartory) Thom & Church in BF. Among 95 substrate classes supplied by Biolog system, the carboxylic acids and methyl esters had the highest utilization extent for the four species, followed by the amino acids and peptides. The descending sequence of carbon sources utilization capability of isolates was *A. sydowii* (52.6%), *M. luteus* (39.5%), *B. sphaericus* (21.6%), and *G. thermoglucosidasius* (17.7%).

Key words: Biolog system; hydrogen sulfide; ammonia; isolates identification; BTF/BF system

Introduction

H_2S and NH_3 are irritating, smelly substances with very low odor thresholds: 0.14 mg/m^3 for H_2S and 32.1 mg/m^3 for NH_3, and different water solubility: 4.12×10^6 mg/m^3 for H_2S and 5.11×10^8 mg/m^3 for NH_3 (Li and Liu, 2004). They are usually simultaneously emitted from various industries or processes, including food processing, rubber processing, fish processing, animal husbandry, compost plants, and wastewater treatment plants, which explain the interest devoted to optimizing their removal (Devinny *et al*., 1999; Chung *et al*., 2005). Excess amounts of H_2S and NH_3 have to be removed for the sake of safety, health and reduction of environmental impacts, such as greenhouse effect, acid rain, and eutrophication.

The biotrickling filter (BTF) and biofilter (BF) have recently been regarded as the best available control technology for odor treatment, if the treatment concentration, the treatment cost of pollutant, and the advantage of completely degrading the contaminants into innocuous or less-contaminating products are considered (Leson and Winer, 1991; Chung *et al*., 2005). Accordingly, some papers have been published recently in the removal of H_2S and NH_3 simultaneously by BTF (Chung *et al*., 2005), BF (Chung *et al*., 2000, 2001; Malhautier *et al*., 2003; Chen *et al*., 2004; Jones *et al*., 2004; Lee *et al*., 2005) or a combination of BTF and BF (Yu *et al*., 2007a, b).

Li and Liu (2004) underlined that bacteria and fungi had a synergistic function so that the hydrophilic and hydrophobic compounds could be efficiently removed by the combined-bioreactor. Therefore, it could be supposed that the combination of BTF and BF should be a good system for the removal of hydrophilic and hydrophobic compounds simultaneously. Yu *et al*. (2007a, b) validated this surmise and applied a BTF/BF system to treat the mixture of H_2S and NH_3 successfully. The results indicated that the acclimation period was shortened 14–18 d compared to the literature (Chung *et al*., 2005), the optimum process conditions were 120 cm for the total height of BTF/BF system and 4.56 L/h for the recycle liquid rate, and the removal mechanisms could be the bio-chain principle and the adsorption-bioregeneration of clinoptilolite (Yu *et al*., 2007a, b). However, there is dearth of the supported information in the biological aspects. Therefore, further studies are still necessary about the predominant isolates identification.

As to biological processes, the predominant isolates were one of the key factors for achieving high removal efficiencies in controlling odors pollution, indicating that the predominant isolates identification is necessary (Chung *et al*., 2005). Many highly efficient deodorizing sulfide bacteria had been isolated and identified, such as *Thiobacillus thioparus* (Malhautier *et al*., 2003), *Thiobacillus thioparus* CH11 (Chung *et al*., 2000), *Pseudomonas putida* CH11 (Chung *et al*., 2001), *Arthrobacter thiooxidans* (Lee *et al*.,

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2005) etc. Similarly, some deodorizing ammonia bacteria had also been found, such as *Nitrosomonas* (Malhautier *et al*., 2003), *Nitromonas europea* (Chung *et al*., 2000), *Arthrobacter oxydans* CH8 (Chung *et al*., 2001), *Arthrobacter thiooxidans* (Lee *et al*., 2005) etc. Biolog system is highly reproducible and widely applied in the identification of isolates based on the ability of the isolates to oxidize 95 different carbon sources (Calbrix *et al*., 2005; Haack *et al*., 1995).

In this study, the BTF/BF system was applied to treat the mixture of H_2S and NH_3. The specific objectives of this research were to identify the predominant isolates, and to analyze microbial sole carbon sources utilization patterns.

1 Materials and methods

1.1 Medium preparation and microbial cultivation

A clinoptilolite was obtained from Tianjingshan Mountain, Henan Province, China. Its characteristics were as follows: specific gravity, 2.16 g/cm^3; rigidity, 3–4; specific surface, 230–320 m^2/g; diameter, 8–10 mm. A kind of wood chip was obtained from Qingdao City, Shandong Province, China, and its characteristics was as follows: length, 2–4 cm; diameter, 0.3–0.5 cm. The clinoptilolite and wood chip were surfactant-modified using hexadecyltrimethylammonium (HDTMA) and epichlorohydrin by the methods described by Li *et al*. (2000) and Zhang and Liu (2004), respectively.

Prior to the experiment, the surfactant-modified clinoptilolite and surfactant-modified wood chip were placed into 30 L activated sludge (Qingdao Haibohe Wastewater Treatment Plant) for 24 h, respectively, and fed low concentration of H_2S and NH_3 simultaneously. Then the surfactant-modified clinoptilolite and surfactant-modified wood chip were packed into BTF and BF, respectively. The detailed results of microbial acclimation can be seen in Yu *et al*. (2007b).

1.2 BTF/BF system and operation

A schematic BTF/BF system used in this study is shown in Fig.1. Both BTF and BT columns with an internal diameter of 20 cm, were 100 cm height and packed with surfactant-modified clinoptilolite and surfactant-modified wood chip to a depth of 80 cm respectively.

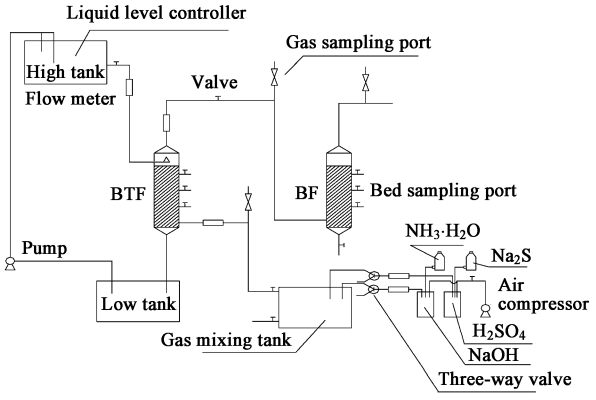

Fig. 1 Scheme of BTF/BF system.

The BTF/BF system was operated in a counter-current mode, i.e., with the gas flowing upward and the recirculation liquid flowing downward. The column wall contained 3 sampling ports (40 cm, 60 cm, 80 cm from the bottom, respectively) for measuring H_2S and NH_3 concentrations and analyzing microbial groups. H_2S and NH_3 (synthetic gas generated from Na_2S and H_2SO_4, $NH_3·H_2O$ and NaOH, respectively), supplied from separate reactors, were introduced into BTF from the bottom by an air compressor and then flowed to the bottom of BF. The H_2S and NH_3 concentrations were controlled by adjusting the concentration of Na_2S and $NH_3·H_2O$ solution and then following a flow meter and a three-way valve (Fig.1). Finally, the purified gas discharged into the environment. A recirculation liquid was pumped from a low level tank with temperature control at 30°C to a high level tank, and uniformly sprayed onto the top of BTF through a nozzle.

One third of the recirculation liquid was replaced every week, and the composition of recirculation liquid as follows (units in mg/L): glucose, 300; $CO(NH_2)_2$, 50; K_2HPO_4, 150; $MgSO_4$, 225; $CaCl_2$, 275; $FeSO_4·7H_2O$, 25.

1.3 Sampling and identification methods

Samples used for the isolates identification were taken from sampling ports at 40 cm of BTF and BF respectively, after the operation reached steady state for 45 d. The isolates used for identification were achieved from the plate streaking method.

Lawns of each isolate were streaked on TSA plates and incubated at 28°C for 24 h. Gram stain results showed the isolates in BTF were Gram positive (GP) bacteria. And they were predominant in quantity. The GP-ROD bacteria, GP-COCCUS bacteria, and FF (Filamentous Fungi) were suspended to a transmittance of 20%, 28% and 75%, respectively. The suspension was then poured into a sterile container, with 150 μl and 100 μl dispensing into the wells of the GP and FF plate, respectively, using an 8-tip micropipette. The GP-ROD, GP-COCCUS and FF plates were then incubated at 35, 30 and 26°C for 24 h, respectively. The detailed identification procedures could be seen in the manufacturer's manual (Biolog Inc., Hayward, CA, USA). The incubation results were put into the Microlog computer program (Biolog system), and a database search was conducted for the closest match.

2 Results and discussion

2.1 Identification and cluster analysis of isolates

The acclimation period and the optimum process conditions of BTF/BF system have been studied (Yu *et al*., 2007a, b). The results indicated that the period of packing biofilm was 10–14 d, which shortened 14–18 d compared to the activated carbon used in the literature (Chung *et al*., 2005), and the removal efficiency for H_2S and NH_3 attained 76.9% and 78.6%, respectively. The optimum height and recycle liquid rate of composite biofilter were 120 cm and 4.56 L/h, respectively (Yu *et al*., 2007a).

The predominant organism groups were a complicated ecosystem including bacteria, protozoa and algae in the biotrickling filter while was only fungi in the biofilter (Yu *et al*., 2007b). Therefore, it is very important to know the structure and metabolic function of the microbial community in a bioreactor to improve its performance. In this experiment, three predominant bacteria were isolated from BTF and one predominant fungi was isolated from BF. By careful observation under a microscope (Olympus 800, Olympus Optical Co Ltd, Japan), two isolates were rod-shaped and straight, (1.0–1.5) μm × (2.0–3.0) μm, and one isolate was spherical, 1.0–1.5 μm in diameter. FF had spores which was similar spherical, (6.5–7.0) μm × (9.5–10.0) μm. The isolates were identified as *Bacillus sphaericus*, *Geobacillus themoglucosidasius* (55°C), *Micrococcus luteus* (ATCC 9341) in BTF and *Aspergillus sydowii* (Bainier & Sartory) Thom & Church in BF by using Biolog system, which showed a 100% similarity of the identified isolates to their database. Biolog identifications were accepted as correct if the similarity index of the genus and species name was 75% or greater at 4 h or 50% or greater at 24 h (Klingler *et al*., 1992), indicating that the identification results in this study were credible. While the identification results are different from the literature (Malhautier *et al*., 2003; Chung *et al*., 2000; Lee *et al*., 2005), this might contribute to the isolates diversity of degrading H_2S and NH_3 or environmental differences of reactors.

The cluster analysis used a mathematical approach to display the relative similarities and differences between isolates based on pattern matching, offering a visual method for examining groups using the linear cluster analysis. The cluster analysis of isolates could be seen in Fig.2, indicating that these identification isolates had a smaller DIST (distance) than other nine microcosms.

Long *et al*. (2000) indicated that *Bacillus sphaerium* played a strong role in nitrogen fixation. Monica *et al*. (2000) discovered that *Micrococcaceae* could degrade effectively nitrite, nitrate and nitrogen oxides. Zhang *et al*. (2005) found that *Aspergillus* could remove nitrogen oxides in the aerobic conditions. Therefore, we could refer that ammonia is removed by the nitrification and denitrification.

The identification results suggest that bacteria and fungi can become the predominant species by controlling the different environment conditions in BTF and BF respectively. Smet *et al*. (1996) and Kennes and Thalasso (1998) reported that seeding a bioreactor with adapted mixed or pure cultures could often enhance the reactor performance or shorten the acclimation or start-up period. Therefore, seeding the identification isolates in BTF/BF system might be feasible for improving the removal efficiency.

2.2 Analysis of sole carbon sources utilization patterns of isolates

On the basis of the database from Biolog system, the sole carbon sources utilization patterns of isolates were formed (Tables 1 and 2). The results indicate that *M. luteus* (ATCC 9341) has the highest utilization extent (52.6%), corresponding to 50 types of different carbon sources, followed by *A. sydowii* (Bainier & Sartory) Thom & Church (39.5%), *G. themoglucosidasius* (55°C) (21.9%) and *B. sphaericus* (17.7%), corresponding to 38, 21 and 17 types of different carbon sources, respectively. Among the substrate classes represented by the highest number of compounds, the carboxylic acids and methyl esters had the highest utilization extent for the four species, followed by the amino acids, peptides. A lower percentage of sugar phosphates than the other substrate classes was utilized, especially by *G. themoglucosidasius* (55°C).

A. sydowii has a relative high carbon sources utilization extent (39.5%), indicating that the isolate is easy to incubate. Zhang *et al*. (2005) observed that *Aspergillus* has a strong degradability to cellulose and lignin. Based

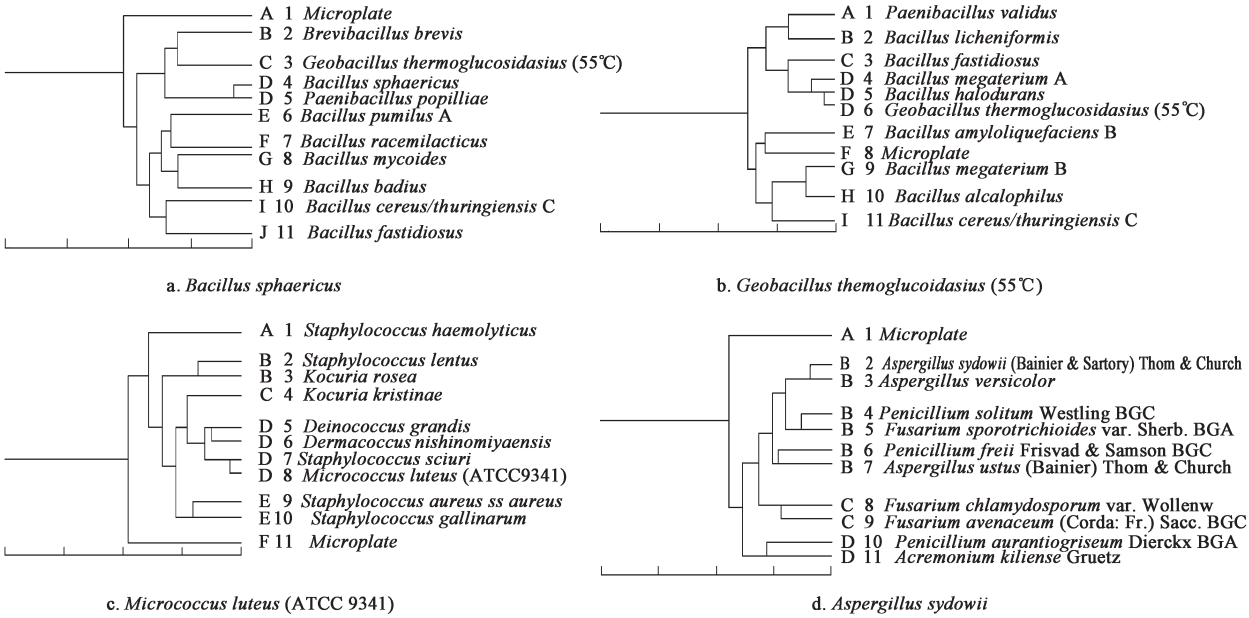

Fig. 2 Cluster analysis of isolates.

Table 1 Sole carbon sources utilization patterns of bacteria

Compound	A	B	C	Compound	A	B	C	Compound	A	B	C
Polymers				β-Methyl-*d*-galactoside	–	–	–	Succinic acid mono-methyl ester	+	+	+
α-Cyclodextrin	–	–	–	3-Methyl-*d*-glucose	–	–	–	Propionic acid	+	+	+
β-Cyclodextrin	–	–	–	α-Methyl-*d*-glucoside	–	–	–	Pyruvic acid	+	+	+
Dextrin	–	+	+	β-Methyl-*d*-glucoside	–	–	–	Succinamic acid	–	–	+
Glycogen	–	–	–	α-Methyl-*d*-mannoside	–	–	–	Succinic acid	+	+	+
Inulin	–	–	–	Palatinose	–	–	+	Amino acides			
Mannan	–	–	–	*d*-Psicose	–	–	–	*n*-Acetyl-L-glutaic acid	–	+	+
Tween 40	–	+	–	*d*-Raffinose	–	–	+	*l*-Alaninamide	–	+	+
Tween 80	+	+	+	*l*-Rhamnose	–	–	–	*d*-Alanine	–	–	+
Sugars and sugar derivatives				*d*-Ribose	–	–	–	*l*-Alanine	–	–	–
n-Acetyl-*d*-glucosamine	–	–	–	Salicin	–	–	–	*l*-Alanyl-glycine	–	–	+
n-Acetyl-β-*d*-mannosamine	–	–	+	Sedoheptulosan	–	–	–	*l*-Asparagine	–	–	–
Amygdalin	–	–	+	*d*-Sorbitol	–	–	–	*l*-Glutamic acid	–	–	+
l-Arabinose	–	–	–	Stachyose	–	–	–	Glycyl-L-glutamic acid	–	–	–
d-Arabitol	–	–	–	Sucrose	–	–	–	*l*-Pyroglutamic acid	–	–	+
Arbutin	–	–	+	*d*-Tagatose	–	–	+	*l*-Serine	–	–	+
d-Cellobiose	–	–	+	*d*-Trehalose	–	–	+	Putrescine	–	–	+
d-Fructose	–	–	–	Turanose	–	–	–	Alcohods			
d-Fucose	–	–	–	Xylitol	–	+	+	2,3-Butanediol	–	–	+
d-Galactose	–	–	+	*d*-Xylose	–	–	+	Glycerol	–	–	+
d-Galacturonic acid	–	–	–	Carboxylic acids methyl esters				Nucleotides and nucleosides			
Gentiobiose	–	–	–	Acetic acid	+	+	–	Adenosine	–	–	+
d-Gluconic acid	–	–	+	α-Hydroxybutyric acid	+	–	+	2′-Deoxy adenosine	–	–	+
α-*d*-Glucose	–	+	–	β-Hydroxybutyric acid	+	+	–	Inosine	–	–	+
m-Inositol	–	–	–	γ-Hydroxybutyric acid	–	–	+	Thymidine	–	–	+
α-*d*-Lactose	–	–	–	*p*-Hydroxy-phenylacetic acid	–	–	+	Uridine	–	–	+
Lactulose	–	–	–	α-Ketoglutaric acid	–	–	–	Adenosine-5′-monophosphate	–	–	+
Maltose	–	–	+	α-Ketovaleric acid	+	–	+	Thymidine-5′-monophosphate	–	–	+
Maltotriose	–	–	+	Lactamide	+	–	+	Uridine-5′-monophosphate	–	–	–
d-Mannitol	–	–	+	*d*-Lactic acid methyl ester	–	–	–	Sugar phosphates			
d-Mannose	–	–	–	*l*-Lactic acid	+	+	+	*d*-Fructose-6-phosphate	–	–	+
d-Melezitose	–	–	+	*d*-Malic acid	–	–	+	*d*-Glucose-1-phosphate	–	–	–
d-Melibiose	–	–	–	*l*-Malic acid	+	+	–	*d*-Glucose-6-phosphate	–	–	–
α-Methyl-*d*-galactoside	–	–	–	Pyruvic acid methyl ester	–	–	+	*d*-L-α-Glycerol phosphate	–	–	–

A: *Bacillus sphaericus*, B: *Geobacillus themoglucosidasius* (55°C), C: *Micrococcus luteus* (ATCC 9341). "+" and "–" present the species has a high or low utilization extent to the carbon source.

Table 2 Sole carbon sources utilization patterns of fungi

Compounds	D	Compound	D	Compound	D	Compound	D
Tween 80	–	Glucuronamide	–	Salicin	–	Sebacic acid	+
n-Acetyl-*d*-galactosamine	–	*d*-Glucuronic acid	+	Sedoheptulosan	–	Succinamic acid	–
n-Acetyl-*d*-glucosamine	+	Glycerol	+	*d*-Sorbitol	+	Succinic acid	+
n-Acety-*d*-mannosamine	–	Glycogen	+	*l*-Sorbose	–	Succinic acid monomethyl ester	–
Adonitol	–	*m*-Inositol	–	Stachyose	–	*n*-Acetyl-*l*-glutamic acid	–
Amygdalin	–	2-Keto-*d*-gluconic acid	+	Sucrose	+	*l*-Alaninamide	–
d-Arabinose	–	α-*d*-Lactose	–	*d*-Tagatose	–	*l*-Alanine	+
l-Arabinose	+	Lactulose	–	*d*-Trehalose	+	*l*-Alanyl-glycine	+
d-Arabitol	–	Maltitol	–	Turanose	+	*l*-Asparagine	+
Arbutin	+	Maltose	–	Xylitol	–	*l*-Aspartic acid	+
d-Cellobiose	–	Maltotriose	–	*d*–Xylose	+	*l*-Glutamic acid	+
α-Cyclodextrin	–	*d*-Mannitol	–	γ-Aminobutyric acid	+	Glycyl-*l*-glutamic acid	+
β-Cyclodextrin	–	*d*-Mannose	–	Bromosuccinic acid	–	*l*-Ornithine	+
Dextrin	–	*d*-Melezitose	–	Fumaric acid	+	*l*-Phenylalanine	+
i-Erythritol	–	*d*-Melibiose	–	β–Hydroxybutyric acid	+	*l*-Proline	+
d-Fructose	–	α-Methyl-*d*-galactoside	–	γ-Hydroxybutyric acid	–	*l*-Pyroglutamic acid	+
l-Fucose	–	β-Methyl-*d*-galactoside	–	*p*-Hydroxy-phenylacetic acid	+	*l*-Serine	+
d-Galactose	–	α-Methyl-*d*-glucoside	–	Ketoglutaric acid	+	*l*-Threonine	–
d-Galacturonic acid	–	β-Methyl-*d*-glucoside	–	*d*-Lactic acid methyl ester	+	2-Aminoethanol	+
Gentiobiose	–	Palatinose	–	*l*-Lactic acid	+	Putrescine	+
d-Gluconic acid	+	*d*-Psicose	–	*d*-Malic acid	+	Adenosine	–
d-Glucosamine	–	*d*-Raffinose	–	*l*-Malic acid	+	Uridine	–
d-Glucose	–	*l*-Rhamnose	–	Quinic acid	–	Adenosine-5′-monophosphate	–
α-*d*-Glucose-1-phosphate	–	*d*-Ribose	+	*l*-Saccharic acid	+		

D: *Aspergillus sydowii*. "+" and "–" present the same meaning as that in Table 1.

on the result, we can infer that wood chip could provide *Aspergillus* with the carbon sources. Therefore, the BF system is easy to operate, since BF does not need the external carbon sources.

Due to a general lack of understanding of the physiological characteristics of the microorganisms, it is important to analyze the sole carbon sources utilization patterns to change the culture conditions. First, replacing glucose by the carboxylic acids and methyl esters which have a higher utilization extent for the predominant isolates in the recirculation liquid, could shorten the acclimation or start-up period of BTF/BF system. Secondly, the enrichment efficiency could be enhanced by designing a selective medium which makes the predominant isolates reproduced rapidly. Furthermore, the pyruvic acid methyl esters which belong to an available but low solubility carbon source, could be added to an enrichment medium in order to enhance the isolation efficiency by producing a transparency circle.

3 Conclusions

In this study, four species, *Bacillus sphaericus*, *Geobacillus themoglucosidasius* (55°C), *Micrococcus luteus* (ATCC 9341) in the BTF and *Aspergillus sydowii* (Bainier & Sartory) Thom & Church in the BF, were found to be able to degrade H_2S and NH_3 (Yu *et al*., 2007a, b). The descending sequence of carbon sources utilization capability of isolates was *A. sydowii* (52.6%), *M. luteus* (39.5%), *B. sphaericus* (21.6%), and *G. thermoglucosidasius* (17.7%). Among the substrate classes represented by the highest number of compounds, the carboxylic acids and methyl esters had the highest utilization extent for the four species, followed by the amino acids and peptides.

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