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The mycobiota of landfill leachates in the pretreatment process in a sequencing batch reactor

Research Article

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Abstract: The paper discusses the dynamics of the accumulation of microscopic fungi, depending on the sludge load (Bx), in activated sludge used for landfill leachate pretreatment. The propagule washout from the sludge into pretreated leachates is determined, including genera and species that may threaten environmental health. An increased accumulation of microscopic fungi in sludge flocs occurred at Bx=0.23-0.45 mg chemical oxygen demand (COD) mg^{-1} d^{-1}. Microscopic fungi were eluted at the maximal Bx value tested of 1.64 mg COD mg^{-1} d^{-1}. Both the activated sludge and the leachate runoff from the sequencing batch reactor (SBR) pose health risks to the environment due to the occurrence of fungi such as *Aspergillus fumigatus, Purpureocillium lilacinum, Cyberlindnera jadinii (C. utilis), Geotrichum candidum* and *G. fragrans*. Their count is sufficient to cause multi-organ infections in homeothermal animals and in humans.

Keywords: *Landfill • Waste • Leachates • Microscopic Fungi • Leachate's treatment • Sbr • Microbiological pollutants*

1. Introduction

Landfill waste is a suitable substrate for the proliferation of saprophytic and pathogenic microorganisms. These microorganisms can access leachates from the landfill waste as well as feces of animals living in landfills (birds, insects, rodents, annelids, *etc.*) [1]. Byeon *et al.* [2] notice that a large part of municipal waste is subject to rot as it is readily colonized by both bacteria and fungi, and, upon the handling of such wastes, these bacteria and fungi can be aerosolized (*i.e.* form bioaerosols). Bioaerosols may present infectious, allergenic or toxic hazards, and, along with volatile organic compounds (VOCs), have been pointed out as agents of special concern. The presence of these microbiological and chemical pollutants can pose a risk to the health of workers in landfills and composting plants, making evident the necessity to control their levels to prevent adverse health effects [3]. Déportes *et al.* [4] have proved that the microbiological hazard arising from fecal contamination is apparently modest, although direct intake of soil contaminated by fecal *Streptococcus* might represent a potential danger. Some of the fungi and bacteria are pathogens or can act through their toxins. The manipulation of compost triggers the aerial dispersion of pathogenic bacteria and fungi that can lead to their inhalation, as shown among workers in composting plants or in mushrooms farms [4] as well as municipal landfills. Albrecht *et al.* [5] have pointed out that *Aspergillus* and *Penicillium* species are some of the most abundant fungi in composting processes.

According to Herr *et al.* [6], gastric symptoms, e.g. nausea, loss of appetite or vomiting, and general health-related complaints are often associated with such contaminants. Possible health effects may also result from medically-relevant bioaerosols released from these sites into neighboring residential areas. In fact, emissions from waste facilities are an issue for

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occupational health and safety as well as environmental hygiene [3]. According to Kummer and Thiel [7], microbial decomposition of organic material occurs under intensified conditions in waste treatment facilities and consequently bioaerosol emissions generated by them present a risk to the environment even though these facilities account only for a small part of a wide variety of potential emission sources.

As well as bioaerosols, microorganisms washed away by residual waters could, together with leachates, contaminate the soil, underground water, penetrate surface waters and migrate over long distances [8,9]. Investigations of soil microbiota conducted by Poputnikova and Terekhova [9] have demonstrated that several functional and structural indices are good markers of modifications occurring in bacterial and fungal communities at various distances from a landfill. Furthermore, deviations of various microbial indices compared to the background (conditionally undisturbed) are considerably different. Poputnikova and Terekhova have pointed out that the influence of the landfill as an environmental pollutant on adjacent soils stimulates the soil microbiota development, growth of bacterial and fungal populations as well as their biomass and biological diversity (shown by the biomass of certain species). In addition, increased spore fungal biomass has been reported and the activation of soil respiration. There is also a high negative correlation between the heavy metals content of the soil and the fungal fraction. Kulig *et al.* [8] observe that the pathogenic bacteria *Listeria monocytogenes* and *Clostridium perfringens,* the saprogenic bacterium *Proteus vulgaris* and microscopic fungi belonging to molds can be used as indicators of leachate sanitary pollution. Microscopic fungi in leachates in five different landfills examined by Kulig *et al.* ranged between 0 and 599 cfu·ml^{-1}. Additional indicators are the total number of mesophilic bacteria and the number of thermotolerant coliform bacteria.

Microscopic fungi are of special importance as bioindicators of sanitary environmental pollution. Chemical compounds present in spores and mycelium showing allergenic effects and many fungal pathogens possess mycotoxins with cytotoxic, mutagenic, neurotoxic and teratogenic properties. Microscopic fungi enter the host organism by inhalation, ingestion and sexually, through the damaged oral mucosa, skin or cornea. They can cause multifocal fungal infections, endocarditis, osteomyelitis, meningitis and other diseases. Immunocompromised persons, *i.e.* in patients with food malabsorption, cancers, autoimmune disorders, AIDS, drug, medication or alcohol addicts and patients with implants are particularly susceptible to fungal infections. Causative organisms of these infections are yeast-like fungi of the genera *Candida*, *Cryptococcus*, *Kloeckera*, *Rhodotorula*, and molds of the genera *Penicillium*, *Aspergillus*, *Pullularia*, *Fusarium*, *Acremonium*, *Paecilomyces*, *Alternaria* and *Culvularia*. *Aspergillus fumigatus* and *Aspergillus niger* show special pathogenicity to humans. The role of microscopic fungi in the transmission of diseases in water is discussed in a study by Grabińska-Łoniewska and Siński [10].

The aim of the study was to determine the dynamics of the accumulation of microscopic fungi in activated sludge used in the landfill leachate pretreatment process in relation to the sludge load. The propagule elution from the sludge into the pretreated leachate was estimated, including genera and species which could present a sanitary threat to the environment. The process was conducted in a sequencing batch reactor (SBR) used to treat sewage by biological methods [11,12].

2. Experimental Procedures

2.1 Samples

Leachate delivered to a collection sump *via* a drainage system near a landfill site designed for municipal waste (*i.e.* waste other than inert and hazardous), in the south-eastern part of the town of Otwock was examined. The site has been in use since 1998 and will operate until 2028. Municipal wastes containing a high amount of biodegradable organic matter exceeding 20 Mg d^{-1} are deposited at the site. The estimated capacity is $12 \cdot 10^5$ Mg. The landfill is lined with a 2 mm polyethylene *high-density (*PEHD) geomembrane. Wastes are stored in districts on 1.5-2 m thick plots. The lining thickness is 0.15 m. Leachate water and landfill gas as well as surface and underground waters near the landfill are monitored [11,13].

2.2 Leachate pretreatment process

Leachate was pretreated in a 6.9 litre SBR equipped with a mixer and a fine bubble aeration system that supplies oxygen concentration of 2 mg O_2 dm^{-3}. The activated sludge from a domestic sewage treatment plant in Piaseczno near Warsaw was used as the seed. Volatile suspended solids constituted 67% of the sludge and mineral solids 33% on average. The sludge concentration in the reactor ranged from 3 to 4 g dm^{-3}, the sludge age (*qx*) was 12.5 days and the hydraulic retention time (*HRT*) was 16 hours. The sludge load (*Bx*) ranged from 0.40 to 1.64 mg COD mg^{-1} d^{-1}, obtained by an increase in the percentage leachate contribution (5, 10, 15, 20 and 30%) in the mixture with synthetic wastewaters prepared according to the recipe by Klimiuk

and Wojnowska-Baryła [14]. The system operated in three 8-hour cycles per day. Each cycle consisted of a 45 minutes fill phase, 30 minutes mixing phase, 2 h 10 min aeration phase, 45 minutes mixing phase, 1 h 5 min aeration phase, 1 h 30 min hour settling phase and 30 minutes decanting phase (including 25 minutes of decanting and 5 minutes idling) [11].

The total number of fungi was determined according to the methodology given by Grabińska-Łoniewska *et al.* [15]. Fungi were isolated with the dilution plate method using the Martin's medium (g · l^{-1}): glucose – 10.0; peptone – 5.0; KH_2PO_4 – 1.0; $MgSO_4 \cdot 7\ H_2O$ – 0.5; agar – 20.0; rose Bengal – 33.3 mg; streptomycin – 30.0 mg and chlorotetracycline – 2.0 mg. Incubation was conducted at 26ºC for 7 days. Fungi counts were given as a mean from three replications in cfu ml^{-1}. The results were converted to dry matter for the activated sludge and given in cfu mg^{-1} d.m.

The genus and species of the fungi were identified based on macro-morphological features using observations of colonies on plates and fungal growth on slides and on micro-morphological features using observations of micro-cultures on agar circles and in water drops (yeasts), biometric measurements (colonies and spores), and biochemical properties (yeasts). Macroscopic studies of molds (filamentous fungi) were conducted on the following media: potato-dextrose agar (PDA, g · l^{-1} of distilled water): potato – 200.0; glucose – 20.0; agar – 20.0; Czapek-Dox (g · l^{-1} of distilled water): $NaNO_3$ – 3; K_2HPO_4 – 1.0; $MgSO_4 \cdot 7\ H_2O$ – 0.5; KCl – 0.5; $FeSO_4 \cdot 7\ H_2O$ - 0.01; saccharose – 30.0; agar – 15.0 and malt extract agar (MEA, g · l^{-1} of distilled water): malt extract – 20.0; agar – 20.0. The colouring (reverse, face), the mycelium structure and the colony size (colony diameter in cm) after a specific period of time were examined. The morphology of reproductive structures: conidia and conidiophores, sporangia and sporangiophores, and the structure of vegetative hyphae (multi- and mono-cellular hyphae) of molds in microcultures on agar circles were observed under a microscope. Yeast colonies on MEA were observed and water drop preparations were made to examine the morphology of yeast cells, asexual reproduction and ascus formation. The formation of the pseudomycelium, arthrospores, blastospores and other reproductive forms of yeasts was observed in cultures on agar plates (PDA, MEA). The ability to assimilate various sugars and nitrates and to produce urease was examined to assess biochemical properties of yeasts. The final classification of molds and yeasts was based on the keys and systematic studies by Domsch *et al.* 1980 [16]; Fassatiova1983 [17]; Kreger-Van Rij 1984 [18]; Kurzman and Fell 2000 [19].

3. Results and Discussion

The microscopic fungi (MF) count in the SBR processed sewage has been found to increase progressively as the percentage of the leachate added to the synthetic domestic sewage increases. When the Bx values ranged from 0.23 to 0.96 mg COD mg^{-1} d^{-1}, the mean MF count removed at the primary treatment reached 90%. When the Bx values ranged from 0.23-0.40 mg COD mg^{-1} d^{-1}, the 375 - 390 cfu ml^{-1} MF count in the inflow decreased to 30-75 cfu ml^{-1} in the outflow. However, when Bx=45 mg COD mg^{-1} d^{-1}, the MF count in the outflow increased approximately tenfold (reaching the mean value of 433 cfu ml^{-1}), and when Bx=0.96 mg COD mg^{-1} d^{-1}, the mean MF count rose to 1410 cfu ml^{-1}. The MF were found to be eluted from the activated sludge when the maximal sewage loading was used (1.64 mg COD mg^{-1} d^{-1}), which was illustrated by an approximately two-fold increase in MF count in the outflow as compared to the inflow. The MF count in the SBR outflow reached the mean value of $60 \cdot 10^3$ cfu ml^{-1} at the above mentioned Bx value.

The analysis of the MF count changes in the activated sludge shows that the MF accumulation rate in the activated sludge flocs is usually similar (*i.e.* from $34 \cdot 10^2$ to $124 \cdot 10^2$, with the mean value of $69 \cdot 10^2$ cfu mg^{-1} of dry matter) when the Bx values range from 0.23 to 0.45 mg COD mg^{-1} d^{-1}. A considerable MF accumulation increase in the sludge was observed at Bx=0.96 mg COD mg^{-1} d^{-1} ($242 \cdot 10^2$ cfu mg^{-1} of dry matter), *i.e.* at the loading preceding the critical value of Bx=1.64 mg COD mg^{-1} d^{-1}. At this Bx value, the MF population in the sludge flocs increased considerably ($576 \cdot 10^2$ cfu mg^{-1} of dry matter) and the MF were eluted rapidly from the site (Table 1; Figures 1, 2).

The sewage microbiota present in the SBR inflow and outflow as well as in the activated sludge was revised taxonomically at Bx values of: 0.45; 0.96 and 1.64 mg COD mg^{-1} d^{-1}. Since the SBR inflow sewage was a mixture of synthetic domestic sewage and natural leachate, the MF species observed in the SBR inflow may be assumed to have been identical with those in the natural leachate of the examined waste material disposal site.

As Table 2 shows, the dominant MF species present in the leachate were: *Aspergillus fumigatus*, *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus*), *Cyberlindnera jadinii* (syn. *Pichia jadinii*) and its anamorphic state *Candida utilis*, *Trichosporon pullulans* (current name *Guehomyces pullulans*), *Geotrichum fermentans,* and some unidentified yeast species. *Aspergillus niger* and *Trichoderma viride* mold species occurred periodically, and so did yeasts and yeast-like fungi: *Saccharomyces cerevisiae*, *Geotrichum candidum* and *G. fragrans*.

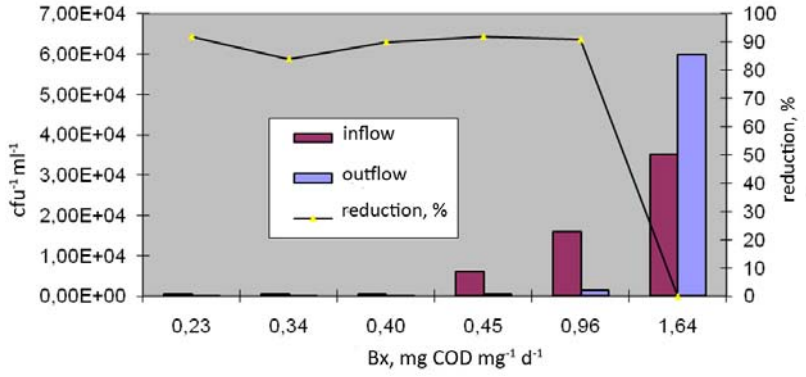

Figure 1. Microscopic fungi in the inflow and outflow and the removal efficiency in the leachate pretreatment process in an SBR.

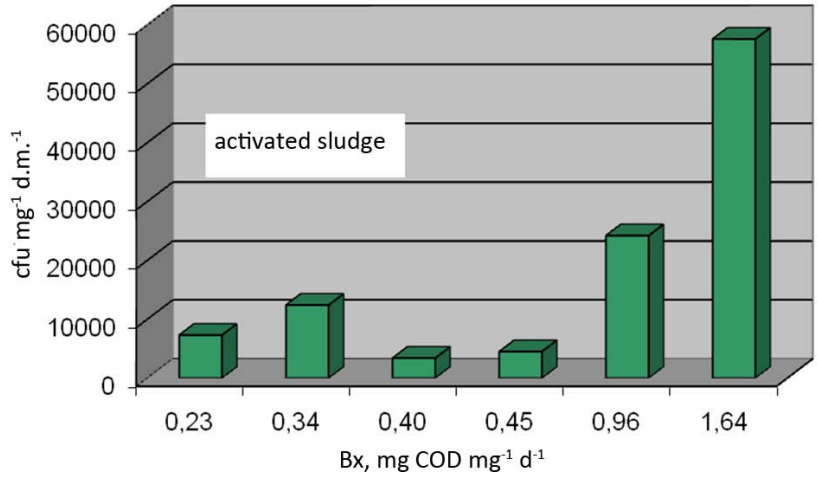

Figure 2. Microscopic fungi in the activated sludge in the leachate pretreatment process in an SBR.

Leachate MF species accumulated in the activated sludge flocs during the treatment process. These included *A. fumigatus, Purpureocillium lilacinum*, *Cyberlindnera jadinii* (*C. utilis*), *Trichosporon pullulans*, *Trichoderma viride*, *C. fermentans*, *G. candidum* and *G. fragrans*, as well as MF species typically not observed in the SBR inflow mixture (probably due to their low count in leachates) such as *A. niger, Mucor plumbeus, Colleotrichum dematium* and *Fusarium* sp.

Numerous MF species occurring in the activated sludge were found in the SBR outflow sewage: *A. niger, Purpureocillium lilacinum*, *Trichosporon pullulans*, *C. utilis*, *G. fermentans* and *G. candidum.* The MF species found neither in the SBR inflow sewage nor in the activated sludge, such as *Cladosporium resinae* and *Dipodascus armillarie*, should be regarded as SBR outflow secondary contaminants.

The MF most identified in the activated sludge and in the SBR outflow sewage are pathogenic or at least potentially pathogenic species. Habitats in the environment and pathogenicity of MF isolated from the leachate inflow and outflow, and from the activated sludge in the SBR are listed in Table 3.

According to the above data, the sewage pretreatment process can be considered to be a sanitary mycological hazard for the natural environment in which it is conducted.

A considerable mycological deterioration of the SBR outflow sewage quality (*i.e.* an MF count increase) was caused by 10% of the leachate added to synthetic domestic sewage (Bx=0.45 mg COD mg^{-1} d^{-1}). A 30% leachate addition to domestic sewage (Bx=1.64 mg COD mg^{-1} d^{-1}) caused MF to be rapidly eluted from the activated sludge flocs. The MF count in the SBR outflow reached $60 \cdot 10^3$ cfu ml^{-1} which can be considered to be a sanitary hazard if a water reservoir is used to receive treated waste water.

What is also alarming is the accumulation of those MF species that are pathogenic or potentially pathogenic

Leachates in the mixture %	*Bx* mgCOD mg^{-1} d^{-1}	Inflow to the reactor cfu ml^{-1}	Activated sludge cfu ml^{-1} cfu mg dm^{-1}	Outflow from the reactor cfu ml^{-1}	Removal in the process %	Mean values: Inflow to the reactor cfu ml^{-1}	Mean values: Activated sludge cfu ml^{-1} cfu mg dm.$^{-1}$	Mean values: Outflow from the reactor cfu ml^{-1}	Mean values: Removal in the process %
1	2	3	4	5	6	7	8	9	10
1	0.23	360	$570 \cdot 10^2$ $138 \cdot 10^2$	40	89	462	$302 \cdot 10^2$	30	92
		564	$35 \cdot 10^2$ 849	20	96		$73 \cdot 10^2$		
3	0.34	460	$370 \cdot 10^2$ $98 \cdot 10^2$	90	80	490	$470 \cdot 10^2$	75	84
		520	$570 \cdot 10^2$ $151 \cdot 10^2$	60	88		$124 \cdot 10^2$		
5	0.40	350	$122 \cdot 10^2$ $34 \cdot 10^2$	40	89	375	$123 \cdot 10^2$	35	90
		400	$124 \cdot 10^2$ $35 \cdot 10^2$	30	92		$34 \cdot 10^2$		
10	0.45	7 000	$180 \cdot 10^2$ $51 \cdot 10^2$	306	96	6 000	$161 \cdot 10^2$	433	92
		5 000	$143 \cdot 10^2$ $40 \cdot 10^2$	560	89		$45 \cdot 10^2$		
20	0.96	15 000	$700 \cdot 10^2$ $217 \cdot 10^2$	1 100	93	16 000	$780 \cdot 10^2$	1410	91
		17 000	$860 \cdot 10^2$ $267 \cdot 10^2$	1 720	90		$242 \cdot 10^2$		
30	1.64	30 000	$2\,000 \cdot 10^2$ $640 \cdot 10^2$	50 000	-	35 000	$1\,800 \cdot 10^2$	60 000	-
		40 000	$1\,600 \cdot 10^2$ $512 \cdot 10^2$	70 000	-		$576 \cdot 10^2$		

Table 1. Microscopic fungi in the inflow, outflow and in activated sludge in the leachate pretreatment process in an SBR depending on the sludge load (Bx).

Sample type	Dominant species	Accompanying species
	Bx=0.45 mg COD $mg^{-1} d^{-1}$	
Inflow	*Aspergillus fumigatus* Fresen. *Candida utilis* (Henneberg) Lodder & Kreger-Van Rij [anamorphic state of *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter] Yeast not identified	*Trichoderma viride* Pers. *Geotrichum fermentans* (Diddens & Lodder) Arx
Activated sludge	*Aspergillus fumigatus* Fresen. *Candida utilis* (Henneberg) Lodder & Kreger-Van Rij [anamorphic state of *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter] *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter (*Pichia jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Kurtzman) *Trichoderma viride* Pers. Yeast not identified	*Colletotrichum dematium* (Pers.) Grove *Fusarium* sp. *Geotrichum candidum* Link *Geotrichum fermentans* (Diddens & Lodder) Arx *Mucor plumbeus* Bonord. *Guehomyces pullulans* (Lindner) Fell & Scorzetti (*Trichosporon pullulans* (Lindner) Diddens & Lodder)
Outflow	*Candida utilis* (Henneberg) Lodder & Kreger-Van Rij [anamorphic state of *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter] *Geotrichum fermentans* (Diddens & Lodder) Arx Yeast not identified	*Guehomyces pullulans* (Lindner) Fell & Scorzetti (*Trichosporon pullulans* (Lindner) Diddens & Lodder)
	Bx=0.96 mg COD $mg^{-1} d^{-1}$	
Inflow	*Geotrichum fermentans* (Diddens & Lodder) Arx *Guehomyces pullulans* (Lindner) Fell & Scorzetti (*Trichosporon pullulans* (Lindner) Diddens & Lodder) Yeast not identified	*Trichoderma viride* Pers. *Geotrichum fermentans* (Diddens & Lodder) Arx
Activated sludge	*Geotrichum fermentans* (Diddens & Lodder) Arx *Aspergillus niger* Tiegh. *Guehomyces pullulans* (Lindner) Fell & Scorzetti (*Trichosporon pullulans* (Lindner) Diddens & Lodder) Yeast not identified	
Outflow	*Geotrichum fermentans* (Diddens & Lodder) Arx Yeast not identified	*Aspergillus niger* Tiegh.
	Bx=1.64 mg COD $mg^{-1} d^{-1}$	
Inflow	*Purpureocillium lilacinum* (Thom) Luangsa-ard, Hywel-Jones & Samson (*Paecilomyces lilacinus* (Thom) Samson) *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter (*Pichia jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Kurtzman) *Trichoderma viride* Pers. Yeast not identified	*Geotrichum candidum* Link *Geotrichum fermentans* (Diddens & Lodder) Arx *Geotrichum fragrans* Morenz *Saccharomyces cerevisiae* Meyen ex E.C. Hansen *Guehomyces pullulans* (Lindner) Fell & Scorzetti (*Trichosporon pullulans* (Lindner) Diddens & Lodder)
Activated sludge	*Aspergillus fumigatus* Fresen. *Candida utilis* (Henneberg) Lodder & Kreger-Van Rij [anamorphic state of *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter] *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter (*Pichia jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Kurtzman) *Trichoderma viride* Pers. Yeast not identified	*Geotrichum candidum* Link
Outflow	*Candida utilis* (Henneberg) Lodder & Kreger-Van Rij [anamorphic state of *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter] *Purpureocillium lilacinum* (Thom) Luangsa-ard, Hywel-Jones & Samson (*Paecilomyces lilacinus* (Thom) Samson) Yeast not identified	*Dipodascus armillariae* W. Gams *Geotrichum candidum* Link *Geotrichum fermentans* (Diddens & Lodder) Arx *Sorocybe resinae* (Fr.) Fr. *Guehomyces pullulans* (Lindner) Fell & Scorzetti (*Trichosporon pullulans* (Lindner) Diddens & Lodder)

Table 2. Species of microscopic fungi occurring in the SBR inflow and outflow and in the activated sludge in the treatment process at Bx=0.23-1.64 mg COD $mg^{-1} d^{-1}$.

for homeothermic animals and humans in the activated sludge, such as *Aspergillus fumigatus, Purpureocillium lilacinum, Cyberlindnera jadinii (C. utilis), Geotrichum candidum and G. fragrans*.

Aspergillus fumigatus is a thermotolerant species which produces carcinogenic mycotixins and fumigacin, an antibacterial antibiotic. It has been repeatedly isolated from the wounds of birds and mammals. It

Species name	Habitats (1) and pathogenicity (2)
Aspergillus fumigatus	(1) soil, municipal waste, compost, guano, nests of birds (2) opportunistic pathogen
Aspergillus niger	(1) soil, water, air, municipal waste, compost (2) saprotroph, occasionally infections in human and animals
Purpureocillium lilacinum (syn. *Paecilomyces lilacinus*)	(1) soil, water, bottom sediments, plumage of birds (2) opportunistic pathogen
Mucor plumbeus	(1) soil, water, guano (2) saprotroph, occasionally may be pathogenic (especially in the warm climate)
Sorocybe resinae (syn. *Cladosporium resinae*)	(1) soil and environments contaminated with petroleum product (2) saprotroph
Colletotrichum dematium	(1) dead plant residues (2) saprotroph, occasionally may be phytopathogenic
Trichoderma viride	(1) soil, plant debris (2) saprotroph
Guehomyces pullulans (syn. *Trichosporon pullulans*)	(1) human respiratory system, water, bottom sediments (2) opportunistic pathogen
Candida utilis [anamorphic state of *Cyberlindnera jadinii*]	(1) human respiratory system (2) opportunistic pathogen
Geotrichum candidum	(1) soil, water, sewage, milk (2) opportunistic pathogen
Geotrichum fermentans	(1) human respiratory system (2) pathogenic to humans
Dipodascus armillariae	(1) dead plant debris and mushrooms (2) saprotroph
Geotrichum fragrans (syn. *Geotrichum fici*)	(1) human respiratory system (2) pathogenic to humans

Table 3. Selected features of the main species of microscopic fungi isolated from leachate inflow, outflow and from the activated sludge in the SBR [16,17, 23-36].

causes tuberculosis-like diseases in the respiratory organs of poultry (*i.e.* pulmonary aspergillosis). It produces mycotoxins such as fumigatin and gliotoxin and causes lung mycosis in humans and mycotoxicosis in cattle [17]. The effect of gliotoxin which is an epipolytidioxopiperazine on the electron transport chain can change according to gliotoxin lateral chains [20].

*Purpureocillium lilacinum (*syn. *Paecilomyces lilacinus)* is a saprotrophic species but it can also be an insect parasite. It is an opportunistic pathogen and can infect homeothermic animals and humans in special cases.

Geotrichum sp. is commonly found in dairy products in which it can eliminate beneficial microorganisms. It is a common saprotroph but it can infect the oral cavity or the respiratory tract as a pathogen [17].

While landfill leachate studies are conducted worldwide, physico-chemical properties and toxicity are mostly analyzed, thus there is a lack of detailed data on microbiological contaminants in leachates [21]. Leachate composition and the unfavorable impact of municipal solid waste dumps on the quality of groundwater are usually examined to determine the amount of leachate produced and its physico-chemical profile as well as to develop leachate treatment methods [8]. Matejczyk *et al.* have isolated small counts of filamentous microscopic fungi from municipal solid waste landfill sites in southern Poland (0-13 cfu ml^{-1}). They found that *Penicillium simplicissimum* was the predominating species (73.2%), followed by *Trichoderma viride* (17.9%). Other species, e.g. *P. citrinum*, *P. purpurogenum* and *Cladosporium sphaerospermum*, occurred less abundantly (from 1.8% to 3.6%) [21]. Kulig *et al.* have proved that leachates from a Polish municipal landfill in Wołomin contain six fungal species: *Aspergillus fumigatus, Penicillium expansum, P. simplicissimum* (syn. *P. janthinellum), P. verrucosum, Trichoderma viride* and *Botryosphaeria stevensii* (syn. *Diplodia mutila)* [8]. Their counts ranged from 50 to 70 cfu ml^{-1}. Grabińska-Łoniewska *et al.* have isolated 13 filamentous species from leachates from a municipal landfill in Otwock. *Trichoderma viride, Clonostachys rosea f. catenulata* (syn. *Gliocladium catenulatum*) and *Mariannea elegans* predominated and ranged between 0-99 cfu ml^{-1} depending on the season [22]. MF count in leachates from the Wola Suchożebrska, Dębe Wielkie and Jaskółowo landfills examined by Grabińska-

Łoniewska *et al.* was 14-20 cfu ml^{-1}, 22-590 cfu ml^{-1} and 465 cfu ml^{-1}, respectively although fungi were not analyzed taxonomically [22].

4. Conclusions

The results lead to the following conclusions:
The MF accumulation increase in the sludge flocs occurred at Bx values ranging from 0.23 to 0.45 mg COD mg^{-1} d^{-1};

The MF growth was stable in the activated sludge and MF were eluted at the maximal Bx value of 1.64 mg COD mg^{-1} d^{-1};

Both the activated sludge and the SBR outflow leachate are a sanitary hazard for the environment due to the presence of MF species such as *Aspergillus fumigatus, Purpureocillium lilacinum, Cyberlindnera jadinii (C. utilis)*, *Geotrichum candidum* and *G. fragrans*. The MF count is sufficient to cause multi-organ infections in homeothermic animals and in humans.

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