Original Research

Qualitative and Quantitative Changes of Potentially Pathogenic Fungi in a Hydrophyte Wastewater Treatment Plant

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Abstract

Undertaken studies on diversity and prevalence of yeast-like fungi aimed at presenting the role of a hydrophyte treatment plant in cleaning municipal wastewater from potentially pathogenic fungi.

One hundred and six isolates of fungi were obtained. The highest diversity of species was found in the sedimentation tank and in I and II reed beds. The dominating species were: *Trichosporon beigelii, Candida albicans, Candida glabrata, Candida dubliniensis, Candida krusei, Candida utilis* and *Candida lipolytica* – isolated also from hospitalized patients. The number of fungal cells decreased progressively from uncountable in the sedimentation tank to 2,060 cfu/dm^3 in the polishing pond.

Reduction of the number and changes in species composition of yeasts like fungi take place in the hydrophyte treatment plants.

Keywords: yeast-like fungi, wastewater, hydrophyte treatment plant

Introduction

Treatment of wastewater and waters, should involve removal of allogenous microorganisms that are a hazard for the health of people and animals. Microorganisms penetrate into waters through sedimentation from atmospheric air or from organic contaminations, particularly municipal wastewaters [1, 2]. They include potentially pathogenic microfungi and can also be treated as widely understood water purity indicators [3-6].

Our work attempts to show the role of an unconventional macrophyte treatment plant in the level of purification of municipal wastewaters. The studies were undertaken concerning the qualitative and quantitative share of yeast-like fungi appearing in the surface layers of water at individual stages of a reed-based treatment plant, with particular focus on species potentially pathogenic for people and animals.

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Materials and Methods

Our investigation was carried out in CW in Nowa Słupia (Central Poland). Sub-surface flow-constructed wetland in Nowa Słupia is an example of the most common type of constructed wetlands in Poland [7]. The wetland was constructed in 1995 in order to treat municipal wastewaters. It is located in the Holy Cross Mountains region (21º05'E–50º52'N) at 250m a.sl. with an annual mean temperature of 8ºC and annual precipitation of 700mm. The wetland consists of: sedimentation pond, areation tank, and three parallel beds (78mx24m) overgrown with *Phragmites* *australis* (common reed). A polishing pond (volume of 750m^3) is used as a final treatment stage. Water from the polishing pond flows to the Słupianka River (class I water quality) (Fig. 1).

The investigations were carried out during in autumn 2006, spring 2007 and summer 2007.

The samples of yeast-like fungi were collected on 17 sites – 2 in the sedimentation pond, 1 in the aeration chamber, 9 on reed beds, 2 in the polishing pond, and 3 in the Słupianka River (Fig. 1). Parallel physical-chemical analyses formed the background for the microbiological tests (Table 1).

The research material consisted of yeast-like fungi isolated from surface water samples. Each water sample (250 ml) was filtered and subjected to diagnostic processes applied in mycology laboratories.

The Millipore membrane filters (diameter 0.47µm) were placed on plates with Sabouraud medium and incubated for 48-72 h at 37ºC, and were used for isolating and counting the fungal colonies' number. Then the colonies were passed on Sabouraud slopes with chloramphenicole and incubated after the passage at 37ºC for 48-72h.

During the individual research seasons the average cell count was calculated. The diagnostics of yeast-like fungi was conducted based on the morphological (micro- and macro-colonies) and biochemical (zymograms and auxanograms) characteristics. The macroscopic assessment was based on characteristics of colonies, while microscopic characteristics in survival preparations were stained with methyl blue and micro-cultures and incubated for 48-72-144 h [8] at 37°C on Nickerson differentiating medium.

The fungi were identified using the keys by Lodder, Kreger-van Rij [9], Midgley et al. [10], de Hoog et al. [11], Kurtzmann, Feel [12] and Howard [13].

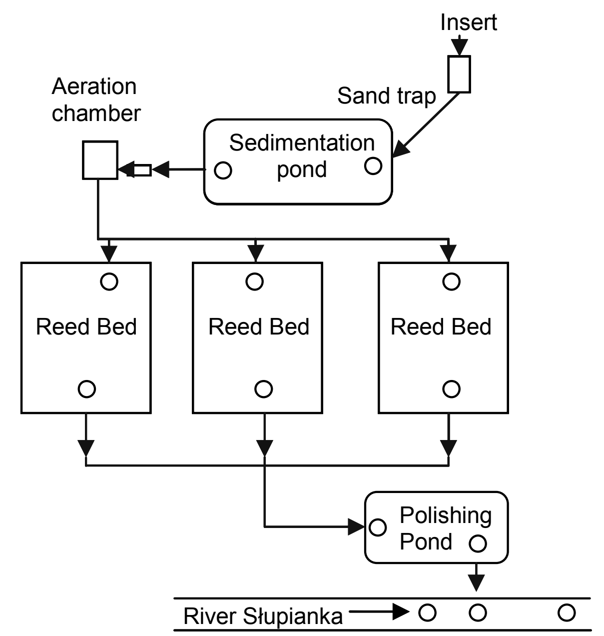

Fig. 1. Scheme of reed wastewater treatment plant in Nowa Słupia.

Photographic documentation was prepared and the strains were catalogued.

Results

During our studies, 106 isolates containing one, two or three species were recovered. The largest diversity of fungal species was found in the sedimentation tank as well as reed beds I and II (Table 2). In those cases potentially pathogenic fungi originating directly from the municipal wastewaters, among others *Trichosporon beigelii, Candida albicans, Candida glabrata, Candida dubliniensis, Candida krusei, Candida utilis* and *Candida lipolytica,* dominated (Table 2). The majority of fungal species were isolated sporadically. However, six species appeared in the samples continually. Those species were: *Candida albicans, Candida guilliermondii, Candida krusei, Candida tropicalis, Candida utilis* and *Saccharomyces cerevisiae*. The fungi were the most numerous in the sedimentation pond (Table 2). Potentially pathogenic fungi of genus *Candida* dominated. The appearance of *Saccharomyces cerevisiae* was recorded during all the research seasons. In the aeration chamber only 5 species were recorded. *Candida krusei* and *Candida utilis* were continually present there. In the surface waters from beds I–III, 13, 15 and 10 species of fungi, respectively, were found. On the first two beds *Candida albicans, Candida dubliniensis, Candida guilliermondi* and *Candida krusei* were always isolated, while in the polishing pond *Rhodothorula glutinis*, the species with purifying properties, was found frequently (Table 2).

The number of cells of fungi isolated from individual sites was gradually decreasing from uncountable in the sedimentation pond to 2060 cfu/dm^3 in the final treatment pond, and 20-30 cfu/dm^3 in the discharge to the Słupianka River. Summer samples from reed bed III were zero as a result of the drying of the pond. The largest numbers of fungi were found during the summer, lower in the autumn and the lowest in the spring. The lowest numbers were found in the samples originating from the discharge to the river and below the discharge to it. The river itself was not free from contamination with potentially pathogenic fungi (Table 3). Above the discharge outlet *Candida guilliermondii*, *Candida krusei* and *Debaryomyces polymorphus* were isolated (Table 2).

Discussion

Municipal wastewaters represent the environment in which, next to organic and inorganic substances dispergated in the water [14], microorganisms harmful to people such as potentially pathogenic fungi are present. They also contain significant quantities of biogenous compounds containing, among other elements, nitrogen, phosphorus and potassium. Pathogenic microorganisms are present in the direct vicinity of the treatment plant and they can represent a biological hazard for people working there [15, 16]. They penetrate into the treatment plant waters through

Table 1. Selected physical and chemical data recorded at consecutive stages in a macrophyte treatment plant.

	pH			Conductivity (μS/cm/s)			BOD_5 (mg/l)			Total nitrogen (mg/l)			Total phosphate (mg/l)			Water temperature (ºC)		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1	6.54	7.10	6.60	1100	1200	1500	767	478	486	177.34	113.64	147.4	32.13	18.10	24.70	-	-	-
2	6.91	6.67	6.74	1200	1200	1600	154	210	169	115.71	96.39	117.80	22.13	16.42	18.17	-	-	-
3	7.12	6.90	6.83	1100	1200	1550	252	156	170	100.07	63.96	107.90	17.46	18.54	20.49	9.1	7.1	16.6
4	6.71	6.61	6.60	1200	1100	1650	160	51	38	101.22	52.00	98.20	18.54	15.22	17.31	9.15	7.15	16.6
5	6.52	6.72	6.81	1100	1150	1560	147	57	29	94.09	49.70	78.90	19.93	13.32	17.20	8.55	7.15	16.7
6	6.74	6.76	6.68	1200	960	1720	69	38	53	86.50	49.93	105.1	19.59	11.12	18.96	-	-	-
7	-	6.83	7.05	-	800	1500	-	12	14	-	38.44	68.56	-	11.02	8.81	-	-	-
8	-	7.39	7.00	-	285	500	-	2	4	-	1.04	11.67	-	0.26	1.46	-	-	-
9	-	7.34	7.07	-	280	300	-	1	1	-	0.91	1.26	-	0.21	0.45	-	-	-

Legend:1 – sedimentation tank, 2 – aeration chamber, 3 – field I, 4 – field II, 5 – field III, 6 – pond, 7 – flow out from the pond, 8 – river below the discharge point, 9 – river above the discharge point, I – 14.11.2006, II – 20.04.2007, III – 21.08.2007.

sedimentation from the air and, from municipal wastewaters, and from people and animals. The fungi most frequently recorded in the treatment plant air, in addition to fungi of genera *Rhodothorula* and *Candida*, also include mould fungi of genera *Aspergillus, Penicillium, Rhizopus* and *Mucor* [15, 16], also recorded in our studies [17].

The presence of potentially pathogenic fungi of genus *Candida*, particularly *Candida albicans*, in fresh municipal wastewater indicates their direct source from the human body, mainly the alimentary system. The fungi isolated from the waters of wastewater treatment plant during own studies could be classified as toilet fungi, which does not exclude their pathogenic properties. The majority of them was earlier isolated from clinical materials obtained from hospitalized patients or people with decreased immunological resistance [11-13, 18-20].

The appearance of yeast-like fungi in surface waters of a macrophytic treatment plant is linked to the wide range of their environmental requirements. The nitrogen compounds, mainly nitrates – products of decay of plants and animals as well as compounds of phosphorus [3, 21] – are the major nutrients for fungi. The wastewater treatment plant waters are characterized by high concentrations of biogens, particularly during the initial stages of treatment, which is linked to their direct flow from municipal sewers. Kurnatowski et al. [22] determined that fungi are characterized by high tolerance to high concentrations of biogenic compounds. Proportionally to the increase in nutrient contents the numbers of fungi increase in the surface waters of the treatment plant. The opposite correlation occurs between the degree of waters saturation with CO_2 and the number of fungi. In the waters of a macrophyte treatment plant the absence of fungi in direct contact with the rizosphere where reactions of both chemical and biological oxidation of various compounds take place was noticed in our own studies. Outside the rizosphere there is a shortage of oxygen or the conditions are anaerobic [23]. It can be assumed, then, that such a decrease in oxygen saturation was accompanied by an increase in the number of yeast-like fungi in the running water. This is consistent with the statement that potentially pathogenic fungi are classified as relative anaerobes [24]. Dilution of organic matter by seasonal water flows that cause good aeration of the ecosystem and increasing the intensity of self-purification of waters causes a decrease in the fungi population [25].

Studies on the presence of fungi in wastewaters have been conducted all over the world for many years. According to Cooke [26], fungi tolerate high levels of pollution even with difficult-to-decompose organic matter. The author recovered the largest numbers of fungi from unprocessed sewage, feces, wastewaters containing residues of decomposing plants and animals, and solid waste of various origins. In the concentrated wastewaters the number of fungal cells per 1ml of water ranged from 132 to 342, in the filtered wastewater it decreased to 10 cells, while Woollett and Hedrick [27] recorded more than 27,000 cells per 100ml of lake water contaminated with municipal and industrial wastewater. Simard and Blackwood [28] observed an obvious increase in the number of fungi in the place of municipal wastewater discharge from the largest cities of Canada to the St. Lawrence River. Similar observations were made by Dynowska [4] analyzing the quantitative and qualitative composition of yeast-like fungi along the Łyna River.

In own studies, high concentrations of nitrogen and phosphorus compounds correlate with the increased population of yeast-like fungi in the sedimentation pond and reed beds. Decreasing physical and chemical parameters allow the conclusion that macrophytic plants can fulfill the

Table 2. Seasonal differences in species of yeast-like fungi recovered from consecutive stages of wastewater treatment at a macrophyte treatment plant.

No.	Species	I	II	III	IV	V	VI	VII	VIII		IX
									VIII-1	VIII-2	
1	*Candida guilliermondii*	+ + +	+	+	+ +	+		+		+	path, sap, ind
2	*Candida krusei*	+ +	+ +	+ +	+	+		+		+	path, sap
3	*Candida glabrata*	+ +	+	+	+		+				path, sap
4	*Candida tropicalis*	+		+	+	+					path, sap, ind, pur
5	*Saccharomyces cerevisiae*	+		+	+	+		+			path, sap
6	*Candida solanii*	+	+								sap
7	*Pichia membranifaciens*	+					+	+			path, sap, pur
8	*Debaryomyces hansenii = Candida famata*	+					+	+			path, sap, pur
9	*Pichia jadinii = Candida utilis*	+ +	+				+				path, sap
10	*Candida mesenterica*	+									sap
11	*Pichia minuta*	+									sap
12	*Candida lipolytica*	+				+					path, sap
13	*Saccharomycodes ludwigii*	+				+					sap
14	*Candida parapsilosis*	+			+			+			path, sap
15	*Trichosporon ovoides*	+			+						path, ind
16	*Dipodascus capitatus = Geotrichum capitatum*	+									path, sap
17	*Trichosporon loubieri*	+									path, sap
18	*Trichosporonoides spathulata*	+									path
19	*Cyniclomyces guttulatus*	+					+				path
20	*Saccharomyces bayanus*	+		+							sap
21	*Candida pulcherrima*			+							sap
22	*Lipomyces lipofer*			+							path, sap
23	*Debaryomyces occidentalis*			+							sap
24	*Zygosaccharomyces bailii*			+							sap
25	*Candida dubliniensis*			+	+						path, ind
26	*Pichia bispora*			+	+						path, sap
27	*Candida albicans*	+ +		+ +	+ + +	+ +					path, ind
28	*Saccharomycopsis capsularis*				+						path, sap
29	*Candida pelliculosa*				+						path, sap
30	*Candida zeylanoides*				+						path, sap
31	*Debaryomyces polymorphus*				+ +		+	+		+	sap
32	*Ambrosiozyma monospora*				+						sap
33	*Debaryomyces maramus*					+					ch, sap
34	*Dekkera bruxellensis*					+					sap
35	*Pichia farinosa*					+			+		sap
36	*Rhodotorula glutinis*						+	+			path, ind, pur
37	*Stephanoascus ciferrii*						+				path, sap
38	*Kluyveromyces lactis var. lactis*							+			sap
39	*Kluyveromyces polysporus*							+			sap
40	*Oosporidium margaritiferum*							+			path, sap
41	*Pichia norvegensis*							+			path

Legend: path - pathogenic, sap – saprophytic, ind - indicator, pur – purifying, [cross-hatched box] - autumn, [light box] - spring, [grey box] - summer

Table 3. The fungal count ranges within individual treatment stages expressed in cfu/dm³.

	Nov.14,2006	Apr.20,2007	Aug.20,2007
sedimentation tank	26,900 - ∞	56,000 - ∞	260,000
aeration chamber	23,800	18,000	66,667
field I	11,233	15,800	14,600
field II	7,067	5,500	3,742
field III	7,033	14,000	0
pond	80	670	4,605
flow out from the pond	60	320	2,060
river below the discharge point	10	30	30
river above the discharge point	40	20	110

Legend: ∞ - uncountable total

function of the filter in the wastewater treatment process [7, 28-31] and for potentially pathogenic fungi [17]. Dynowska [3] reports that the largest populations of yeast-like fungi appeared in the autumn in the littoral of lakes and astatic reservoirs with higher plants. The author suspects that the large population of fungi could be correlated to substances excreted by those plants at the final stage of vegetation process or with the death of those plants. In our own studies conducted in a macrophytic treatment plant an increase in the population of fungal cells in reed fields was also recorded during early autumn.

After the water has flown through reed fields, in the final purification pond species appeared that confirmed self-purification of water such as *Rhodotorula glutinis* and *Debaryomyces hansenii*. This means that the macrophyte treatment plant fulfills its role in treatment of water intended for discharge to the Słupianka River. The river itself is not free from contamination with potentially pathological fungi that it carries with the waters from its upper section. *Candida guilliermondii* was recovered above the discharge. In the water flowing from the sedimentation pond, on the other hand, the presence of *Candida guilliermondii, Candida krusei, Debaryomyces polymorphus* and *Debaryomyces hansenii* was confirmed. This, however, is of no major importance in the overall assessment of the condition of flowing waters as below the discharge only *Pichia farinosa* was recorded.

Fungi possessing "purifying" properties were isolated also from the unit of biocenoses populating the reactors for heterotrophic denitrification of wastewaters. They included *Candida tropicalis, Pichia minuta, Rhodotorula glutinis, Rhodotorula rubra, Geotrichum candidum* and *Trichosporon cutaneum*. The above-mentioned fungi are able to metabolize acetic and lactic acids, methanol, phenol, 4-chlorophenol and glycerol, using them as a source of carbon removing by the same those harmful substances from wastewaters [32-37]. In macrophyte treatment plants, complex nitrogen transformation processes (amonification, nitrification and denitrification) occur in the direct neighborhood, while in the conventional treatment plants those processes are separated both in space and time [23].

In reed-based treatment plants complete reduction and purification of waters from fresh municipal wastewaters takes place. That is confirmed by the results of mycological tests that are of no less importance in microbiological assessment of waters than bacteriological tests [38, 39].

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