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The Occurrence of Fungi in Activated Sludge from MBRs

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***Abstract*—**The objective of this study is to evaluate the occurrence of fungi in aerobic and anoxic activated sludge from membrane bioreactors (MBRs). Thirty-six samples of both aerobic and anoxic activated sludge were taken from 2 MBR treating domestic wastewater. Over a period of eight months 2 samples from each plant were taken per month. The samples were prepared for count and definition of fungi. The obtained data show that, sixty species belonging to 27 genera were collected from activated sludge samples under aerobic and anoxic conditions. Regarding to the fungi definition, under aerobic condition the *Geotrichum* was found at (8.8%) followed by *Penicillium* (75.0%), *Yeasts* (65.7%) and *Trichoderma* (55.5%), while *Yeasts* (77.1%) *Geotrichum candidum*and *Penicillium* (61.1%) species were the most prevalent in anoxic activated sludge. The results indicate that activated sludge is habitat for growth and sporulation of different groups of fungi, both saprophytic and pathogenic.

***Keywords*—**Aerobic conditions, Anoxic conditions, Activated sludge, Membrane bioreactor, Fungi.

I. Introduction

THE activated sludge process is widely used for treating wastewater, process stability and final effluent quality largely depends upon the composition of the biomass in activity sludge plant. Operational problems such as bulking and scum formation occur when the microorganisms are dominating the sludge population. Microscopic sludge investigation is therefore a necessity for process control and stable plant operation [1].

Abdel-Hafez and El-Sharouny (1990) [2] reported that, the numerous microorganisms are almost present in large numbers in sewage or soil amended with activated sludge. Several fungi previously isolated from these substrates are known to be pathogenic to plants, animals and human. Since many fungi have been found to be the agents responsible for human mycoses [3], since the fungi occur in abundance in sewage sludge and amended soils, studies on fungal incidence in these environments are of hygienic, epidemiological and ecological significance. Besides sludge is increasingly being used to fertilize agricultural and forest areas and to reclaim devastated terrains. Hence, the recognition of the distribution of pathogenic fungal species in such activated sludge is important [4].

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The activated sludge process comprises 2 liquid stream processing units-the aeration basin (biological reactor) and the secondary clarifier. The aeration basin provides the environment for transformation and removal of pollutants by a mixed variable consortium of micro- and macro-organisms termed activated sludge. The micro-organisms include eubacteria, filamentous bacteria, algae, fungi, protozoa and rotifers. Flocs are the basic ecological units of activated sludges. Fungal hyphae are often associated with flocs, but rarely predominate under normal operating conditions [5].

Although it is accepted that micro-organisms are directly responsible for the effectiveness and success of the activated sludge treatment process, the complexity of microbiological populations is often under-estimated during design of the latter. Full understanding of the ecological, physiological and biochemical activities of the microflora is necessary for optimal control of the process [6]. Waste waters with their high organics content are a suitable medium for a great number of microorganisms among which there appear at times also some species of fungi [7].

The aim of this study was the isolation and identification of fungi in activated sludge with MBRs from two wastewater treatment plants through 8 months in Berlin, under different conditions

II. Materials and Methods

Thirty-six activated sludge samples were collected from each aerobic and anoxic wastewater treatment plants with MBRs in two places in Berlin (Vera in wedding and BWB Margaretenhöhe). Two samples monthly, during a period of eight months (from August/08 to May/09) from each plant were taken. Samples were put in clean and sterile bottles sealed and transferred to the laboratory where fugal analysis was made. Aliquots of 0.1 ml homogenized activated sludge [1] were plated on rose Bengal chloramphenicol agar (RBCA), three plates were used for each samples. Plats were incubated at 30oC for 1-2 weeks to allow for development of pigment on colonies to facilitate complete differentiation of fungal types. Repeated subculturing on RBCA was necessary to obtain pure cultures. Isolates were characteristics such as pigmentation of the mycelium and direction of growth of the hypha, whether aerial or lateral, microscopic observation of structures involved in asexual reproduction e.g., conidia or spores, and in sexual reproduction, and the presence of fruiting bodies. For the identification of fungal species the following references were used ([8]; [9]; [10]; [11]; [12]; [13]; [14]).

III. Results

A. Fungi Recovered from Aerobic Activated Sludge Samples

Forty-eight species representing 27 genera were collected from 36 samples on rose Bengal chloramphenicol agar (RBCA) at 30 oC for 1-2 weeks (Table 1). *Geotrichum* presented by *G. candidum*) was the most common genus and was recovered in high frequency of occurrence (88.8%) of samples. *Penicillium* occupied the second place in the number of cases of isolation and was recovered from 75.0% of samples. It was represented by 10 species of which *P. citrinum* was isolated in moderate frequency and emerged in 30.5% of the samples matching 40.74% of total *Penicillium*.

Yeast occupied the third place in the number of cases of isolation and was recovered from 65.7% of the samples. *Trichoderma* occupied the Fourth place in the number of cases of isolation and was recovered from 55.5% of all activated sludge sample. From the genus 2 species were identified. *T. hamatum* and *T. virida* were recovered from (36.3 and 22.2%) of all samples, respectively. *Aspergillus* was recovered from 47.2% of all samples. It was represented by 8 species of which *A. flavus* was isolated in moderate frequency. It emerged in 25.0 % of the samples matching 52.94% of total *Aspergillus*. *Doratomyces* (represented by *D. stemonitis*) was recovered 47.2% of all aerobic activated sludge samples. *Candida* (represented by C. albicans) was recovered from 41.6% of all samples. *Gymnoascus* (represented by *G. reesii*) was recovered from 41.6% of the samples.

Rhodotorula (represented by *R. rubra*) was recovered from 33.3% of the samples. *Fusarium* (represented by *F. dimerum* and *F. solani*) was recovered from 27.7% of the samples. *F. dimerum* was isolated in low incidence, emerging in 13.8% of samples matching about 50.0% of total *Fusarium*. *Alternaria* (represented by *A. chlamydospora*) was isolated in low frequency and comprised 25.0% of all samples.

Gibberella (represented by *G. accuminata*, *G. avenacea* and *G. fujikuroi*) was isolated in low frequency and comprised 13.8% of all samples. Mucor circinelloides was isolated in low frequency and comprised 13.8 % of all samples. *Trichophyton* (represented by *T. ajelloi* and *T. equinum*) was isolated in low frequency and comprised 8.3% of all samples. *Chrysosporium tropicum*, *Cladosporium cladosporioide*, *Embellisia didymospora*, *Paecilomyces lilacinus*, *Rhizopus arrhizus* and *Stachybotrys elegans*, were isolated in rare frequency and comprised 5.5% of the samples matching, respectively. *Aurobasidium pullulans*, *Gliocladium roseum*, *Myrothesium cinctrum*, *Oidiodendron griseum*, and *Scopulariopsis brevicaulis* , were isolated in rare frequency and comprised 2.7% of the samples.

B. Fungi Recovered from Anoxic Activated Sludge Samples:

Forty-one species belonging to 20 genera were collected from anoxic sludge samples during this investigation (Table 2). Yeast was the most common genus and was recovered in high frequency of occurrence (77.1%) of samples. *Geotrichum candidum* was occupied the second place in the number of cases of isolation and was recovered from 75.0% of the samples. *Penicillium* was the Third place in the number of cases of isolation and recovered from 61.0% of the samples. It was represented by 10 species of which *P. citrinum* was isolated in low frequency and emerged in 41.6% of the samples matching 68.18% of total *Penicillium.*

TABLE I NUMBERS OF CASES OF ISOLATION (OUT OF 36), PERCENTAGE FREQUENCY AND OCCURRENCE REMARKS OF FUNGAL GENERA AND SPECIES RECOVERED FROM AEROBIC ACTIVATED SLUDGE IN MBRS FOR ROSE BENGAL CHLORAMPHENICOL AGAR (RBCA) MEDIA AT 30°C

Genera & species	NCI	%F	OR
Alternaria chlamydospora Mouchacca	9	25.0	M
Aspergillus	17	47.2	M
A. candidus Link	1	2.7	R
A. flavus Link *var. flavus*	9	25.0	M
A. flavus Raper & Fennell *var. columnaris*	2	5.5	R
A. fumigatus Fresenius	8	22.2	L
A. niger van Tieghem	6	16.6	L
A. ochraceus Wilhelm	3	8.3	R
A. terreus Fennell & Raper *var. terreus*	1	2.7	R
A. versicolor (Vuillemin) Tiraboschi	1	2.7	R
Aurobasidium pullulans	1	2.7	R
Candida albicans (Robin)Berkh	15	41.6	M
Chrysosporium tropicum Carmichael	2	5.5	R
Cladosporium cladosporioide (Fresenius) de Vries	2	5.5	R
Doratomyces stemonitis (Persoon) Morton & Smith	17	47.2	M
Embellisia didymospora (Eidam) Vuillemin	2	5.5	R
Fusarium	10	27.7	M
F. dimerum Penzig	5	13.8	L
F. solani (Mart.) App. et Wr	6	16.6	L
Geotrichum candidum Link	32	88.8	H
Gibberella	5	13.8	L
G. accuminata Wollenweber	2	5.5	R
G. avenacea R.J. Cook	1	2.7	R
G. fujikuroi (sawada) Wollenweber *var. fujikuroi*	3	8.3	R
Gliocladium roseum Bainier	1	2.7	R
Gymnoascus reesii Baranetzky	15	41.6	M
Mucor circinelloides van Tieghem	5	13.8	L
Myrothesium cinctrum (Corda) Saccardo	1	2.7	R
Oidiodendron griseum Robak	1	2.7	R
Paecilomyces lilacinus (Thom) Samson	2	5.5	R
Penicillium	27	75.0	H
P. brevicompactum Dierckx	6	16.6	L
P. chrysogenum Thom	7	19.4	L
P. citrinum Thom	11	30.5	M
P. corylophilum Dierckx	3	8.3	R
p. duclauxii Delacroix	1	2.7	R
P. funiculosum Thom	1	2.7	R
P. glabrum (Wehmer) Westling	2	5.5	R
P. oxalicum Currie & Thom	1	2.7	R
P. roquefortii Thom	3	8.3	R
P. verrucosum Dierckx *var verrucosum*	1	2.7	R
Rhizopus arrhizus Fischer	2	5.5	R
Rhodotorula rubra (Jorgensen) FC Harrison	12	33.3	M
Scopulariopsis brevicaulis (saccardo) Bainier	1	2.7	R
Stachybotrys elegans (Pidopl.) W. Gams	2	5.5	R
Trichoderma	20	55.5	H
T. hamatum (Bonorden) Bainier	13	36.3	M
T. virida Persoon	8	22.2	L
Trichophyton	3	8.3	R
T. ajelloi (Vanbreuseghem) Ajello	2	5.5	R
T. equinum Malmsten	1	2.7	R
Yeasts	23	65,7	H

NCI = Number of cases of isolation (out of 36), %F = Percentage frequency of occurrence (calculated per 36 samples) and OR = Occurrence remarks: [H= High occurrence, isolated more than 18 cases (out of 36 samples), M= Moderate occurrence, from 9 to 18 cases, L = Low occurrence, from 5 to 8 cases and R = Rare occurrence, less than 5 cases.

Aspergillus was isolated in moderate frequency and comprised 41.6% of the samples. From the genus 6 species were identified of which *A. flavus* was recovered in 19.4% of frequency of all samples matching 46.6% of total *Aspergillus*. *Doratomyces stemonitis* was recovered from 30.5% of all samples. Trichoderma was isolated in moderate frequency and comprised 30.5% of all sludge samples. From the genus 3 species were identified *T. hamatum, T. koningii* and T. virida. They emerged in 54.4, 27.3 and 11.1% of the samples matching 8.9, 2.73 and 36.4 % of total *Trichoderma*, respectively. *Candida* (*C. albicans*) and *Gibberella* (*G. accuminata* and *G. fujikuroi*) were isolated in moderate frequency occurrence matching collectively 27.7% of samples. *Fusarium* (*F. dimerum, F. oxysporium* and *F. solani*) and *Rhizopus* (*R. arrhizus* and *R. oryzae*) were isolated in low frequency occurrence matching collectively 16.6% of samples. *Gymnoascus* (*G. reesii*) was recovered 8.3% of all samples.

Alternaria (*A. alternate* and *A. chlamydospora*) and *Scopulariopsis* (*S. brevicaulis*) were isolated in rare frequency and comprised 5.5% of all samples. *Cladosporium oxysporium, Mucor circinelloides, Paecilomyces variotii, Rhinocladiella atrovirens, Stachybotrys elegans, Trichophyton terrestre* and *Trichosporon pullulans* were isolated in rare frequency and comprised 2.7% of the anoxic activated sludge samples.

IV. Discussion

The results indicate that activated sludge is habitat for the growth and sporulation of different groups of fungi, both saprophytic and pathogenic. It was clear that, the wastewater treatment in MBRs had an effect on the number of colony forming for both aerobic and anoxic activated sludge. Fifty eight species belonging to 28 genera were collected in this study from activated sludge samples under aerobic condition (26 genera and 46 species) and anoxic condition (20 genera and 41 species). There is basic similarity between mycobiota of aerobic and anoxic activated sludge with the most frequency fungi were *Yeast, Geotrichum, penicillium* and *Trichoderma*.

Some fungi were recovered from aerobic activated sludge only as *Aspergillus candidus, A. flavus var columnaries, A. terrus var terrus, A. versicolor, Aurobasidium pullulans, chrysosporium tropicum, Cladosporium cladosporiods, Gibbrella accuminata, Gliocladium roseum, Myrothesium circtrum, Oidiodendron griseum, Paecilomyces lilacinus* and 3 species from *Penicillium* (*P. duclauxii, P. funiculosum* and *P. verrucosum*), *Rhodotorula rubra, Syncephalastrum racemosum, Trichophyton ajelloi* and *T. equinum*. On the other side some fungi were isolated only from anoxic samples as, *Alternaria alternate*, 2 species from *Aspergillus* (*A. terrus var Africans* and *A. sydowii*), *Cladosporium oxysporium, Fusarium oxysporium, Paecilomyces variotii*, 3 species from *Penicillium* (*P. expancum, P. puberelium* and *P. vinaceum*), *Rhinocladiella altroviren, Rhizopus oryzae, Trichoderma koningii, Trichophyton terrestre* and *Trichosporon pullulans* (Table 1 and 2).

These results were almost agreed to some extent with the finding reported by [4]; [15]; [16-17]; [18-19]; [20]; [21]; [22]; [23-2]; [24] and [25].

Most of fungal isolates in our study such as Alternaria, Aspergillus, Geotrichum, Microsporium, Paecilomyces, Stachybotrys and Trichophyton, are well known a pathogenic or potentially pathogenic fungus and recovered from human dermal lesions ([26]; [27]; [28] and [29]).

Table II Numbers of cases of isolation (out of 36), percentage frequency and occurrence remarks of fungal genera and species recovered from anoxic activated sludge in MBRs for rose bengal chloramphenicol agar (RBCA) media at 30°C

Genera and species	NCI	%F	OR
Alternaria	2	5.5	R
A. alternate (Fries) Keissler	2	5.5	R
A. chlamydospora Mouchacca	1	2.7	R
Aspergillus	15	41.6	M
A. flavus Link *var. flavus*	7	19.4	L
A. fumigatus Fresenius	3	8.3	R
A. niger van Tieghem	2	5.5	R
A. ochraceus Wilhelm	1	2.7	R
A. terreus Thom *var. africanus*	2	5.5	R
A. sydowii (Bainier & Sartory) Thom & Church	1	2.7	R
Candida albicans (Robin)Berkh	10	27.7	M
Cladosporium oxysporium Berkeley & Curtis	1	2.7	R
Doratomyces stemonitis (Persoon) Morton & Smith	11	30.5	M
Fusarium	6	16.6	L
F. dimerum Penzig	2	5.5	R
F. oxysporium Schlechtendal	1	2.7	R
F. solani (Mart.) App. et Wr	4	11.1	R
Geotrichum. candidum Link	27	75.0	H
Gibberella	10	27.7	M
G. accuminata Wollenweber	4	11.1	R
G. fujikuroi (sawada) Wollenweber *var. fujikuroi*	8	22.2	L
Gymnoascus reesii Baranetzky	3	8.3	R
Mucor circinelloides van Tieghem	1	2.7	R
Paecilomyces variotii Bainier	1	2.7	R
Penicillium	22	61.1	H
P. brevicompactum Dierckx	3	8.3	R
P. chrysogenum Thom	7	19.4	L
P. citrinum Thom	15	41.6	M
P. corylophilum Dierckx	4	11.1	R
P. expansum Thom	1	2.7	R
P. glabrum (Wehmer) Westling	2	5.5	R
P. oxalicum Currie & Thom	1	2.7	R
P. puberulum Bainier	1	2.7	R
P. roquefortii Thom	3	8.3	R
P. Vinaceum Gilman & Abbott	1	2.7	R
Rhinocladiella atrovirens Nannf	1	2.7	R
Rhizopus	6	16.6	R
R. arrhizus Fischer	2	5.5	R
R. oryzae Went & Prinsen-Geerligs	4	11.1	R
Roheodtorula rubra (Jorgensen) FC Harrison	15	41.6	M
Scopulariopsis brevicaulis (saccardo) Bainier	2	5.5	R
Stachybotrys elegans (Pidopl.) W. Gams	1	2.7	R
Trichoderma	11	30.5	M
T. hamatum (Bonorden) Bainier	6	16.6	L
T. koningii Oudemans	3	8.3	R
T. virida Persoon	4	11.1	R
Trichophyton terrestre Durie & Frey	1	2.7	R
Trichosporon pullulans (Linder) Diddens & Lodder	1	2.7	R
Yeasts	27	77.1	H

V. Conclusion

On the conclusion, activated sludge produced from MBRs is rich in moulds and other dermatophytes. Most fungi were recovered in the present investigation can be considered as a potential pathogen. Therefore, all workers in the field of activated sludge process, wastewater treatment and farm operation should be careful to avoid mycotic infections and the productions must be adapted to control the spread of pathogenic fungi in the environment. Also these experiments illustrate the possible health risk problems that may arise in the use of sludge for land reclamation and fertilization.

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