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# Comparative study on fungal communities of full scale municipal and industrial wastewater treatment plants

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## ABSTRACT

Fungi are eco-friendly agents and significant contributors to the various functions of activated sludge and can be as efficient as bacteria in the removal of organic matters from wastewater; however, how the types of wastewater and operating conditions affect the fungal communities are remained unexplored compared to bacteria. In this study, a culture-dependent method was used to assess the fungal diversity in three full-scale plants treating petrochemical, acrylonitrile butadiene styrene (ABS) resin manufacturing and municipal waste waters. Moreover, some physiochemical factors and operational parameters were determined. A total of 256 strains belonging to nine genera of *Penicillium, Chrysosporium, Aspergillus, Trichoderma, Trichothecium, Cladosporium, Fusarium, Acremonium,* and *Geotrichum* were isolated. Among them, *Geotrichum, Cladosporium, Aspergillus, Penicillium, Chrysosporium* and *Trichoderma* were the most frequent populations isolated from the wastewater treatment plants (WWTPs). The results indicated that the types of wastewater, as well as the operating conditions are the important factors influencing fungal community structure and abundance.

*Keywords:* Activated sludge; Wastewater; Fungal community; Petrochemical; ABS

## 1. Introduction

Biological treatment using activated sludge process is widely used to reduce organic constituents from both municipal and industrial waste waters [1–6]. There are many parameters affecting the performance of activated sludge process including the nature of wastewater contaminants, environmental conditions and microbial communities, interactions, and functions within the bioreactor [7–9].

A wide range of microorganisms including bacteria, fungi, protozoa, and algae play a key role in degrading organic contaminants in biological treatment process [10–12]. An environmentally friendly, cost-effective and high efficient reduction of organic contaminants is achievable using microorganisms especially bacteria and fungi in the biological wastewater treatment systems [13]. Although bacteria are the main members of the activated sludge, there are some advantages that make fungi as prior as other microorganisms. These organisms do not grow fast like bacteria, but they adapt to the environment more easily and are capable to grow in extreme conditions in light of the nutrient deficiency,

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low pH, high salinity, high chemical oxygen demand (COD), etc. [10,14,15]. Like bacteria, fungi play an important role in the degradation of complex and toxic substances and reduction of organic matters [11,16,17]. However, fungal community structure and functions in the activated sludge have so far been neglected compared to bacteria [18]. Therefore, it is necessary to identify the dynamics of the fungal communities residing in the activated sludge to obtain essential information about their use in wastewater treatment.

Isolation and identification of fungal strains from industrial and municipal wastewater treatment plants (WWTPs), and contaminated soil/sediment samples have been well documented in the literature [1,19–22]. On the other hand, biodegradation potential of specific contaminants such as phenolic compounds [17,19,23], petroleum hydrocarbons [7,24] and polycyclic aromatic hydrocarbons (PAHs) [21,23,25] have been studied using various fungal strains. Since, there is no comparative study in the literature about fungal communities involved in activated sludge treating petrochemical, acrylonitrile butadiene styrene (ABS) and municipal waste waters, this study was conducted to describe and compare the distribution of fungi in the monitored full-scale WWTPs. Other aims of this study were the characterization of the waste waters and the influence of operating conditions on fungal diversity and abundance in the studied WWTPs.

## 2. Materials and methods

### *2.1. Description of WWTPs*

This study was carried out at a petrochemical (PWWTP), an ABS resin manufacturing (ABS-WWTP), and a municipal (MWWTP) wastewater treatment plants operated in Tabriz, Iran. The summary of design characteristics of the plants is presented in Table 1.

The PWWTP is designed to treat wastewater generated from industries including polymeric and non-polymeric units, and ABS effluents (after pretreatment at ABS-WWTP) within the petrochemical complex. The plant consists of screens, API separator, equalization basin, coagulation and flocculation, dissolved air floatation (DAF) system, aeration tanks (conventional extended aeration), primary clarifier, coagulation and secondary clarifier along with the filtration units. The ABS-WWTP also operates to pretreat the ABS effluents, and the out stream of the plant flows to the PWWTP. This plant comprises of fine screens, grit chambers, equalization basin, DAF system, aeration tanks (conventional activated sludge) and clarifiers. Finally, the MWWTP is designed to treat municipal wastewater of Tabriz city, and consists of coarse screens, grit chambers, primary clarifiers, aeration tanks (conventional activated sludge), final clarifiers, and disinfection units. The schematic flow diagrams of evaluated WWTPs are illustrated in Fig. 1.

### *2.2. Chemicals*

Bacteriological grade sabouraud dextrose agar (SDA), rose bengal agar (RBA), and reasoner's 2A agar (R2A) were purchased from Merck and Hi-Media Companies. All other materials used for chemical and biochemical analysis were analytical grade and obtained from the Merck.

### *2.3. Sample collection and analytical procedures*

Samples were collected during six campaigns from the June, 2014 to February, 2015. During this time, 12-h composite samples were taken from the inlet and outlet of WWTPs as well as the inlet and outlet of biological units then preserved in 4°C until measurements which were done as per the standard procedures [26]. At each sampling run, one liter of suspended activated sludge was taken under aseptic conditions in sterile 1500 mL glass bottles from the desired points of aeration tanks and kept on ice during transportation to the laboratory intended for microbiological analysis.

Dissolved oxygen (DO), temperature and pH were measured in-situ by DO-meter (Aqualytic AL20OXi), and Microprocessor pH-meter (EDT-RE-357), respectively. The wastewater key parameters including COD, biochemical oxygen demand ($BOD_5$), total suspended solids (TSS), phosphate ($PO_4^{3-}$), ammonia-N, nitrate, oil and grease, phenol, cyanide, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and sludge volume index (SVI) were measured based on the standard methods for the examination of water and wastewater [26].

### *2.4. Isolation and identification of microbial community*

Sample suspensions were prepared by homogenizing 10 mL of each sample in 90 mL of phosphate buffer solution (8 g NaCl, 0.2 g KCl, 3.53 g $Na_2HPO_4{\cdot}12H_2O$ and 0.27 g $KH_2PO_4$, in 1 L of distilled water; pH 7.2), and serially diluted (6-fold) in the same phosphate buffer solution. The fungi were grown using 2 culture media including SDA and RBA. To prevent the bacterial growth, 0.1 g/L chloramphenicol was added to the RBA and SDA media. The cultures were incubated for 7 d at 25°C. After the incubation period, colonies were selected from the cultivated plates and then transferred to the SDA medium to obtain pure cultures. Fungi were identified according to the general principles of fungal classification using slide culture and lactophenol cotton blue (LPCB) staining followed by the

Table 1
Summary of the design parameters of WWTPs

| WWTP name | Process type | Type of reactor | Nominal capacity ($m^3$/d) | | HRT* (Aeration unit) (h) |
|---|---|---|---|---|---|
| | | | Min | Max | |
| PWWTP | Extended aeration | Plug flow | 4800 | 5520 | 27.5 |
| ABS-WWTP | Complete mix | CMAS** | 800 | 1000 | 22.5 |
| MWWTP | Complete mix | CMAS | 129600 | 216000 | 4–8 |

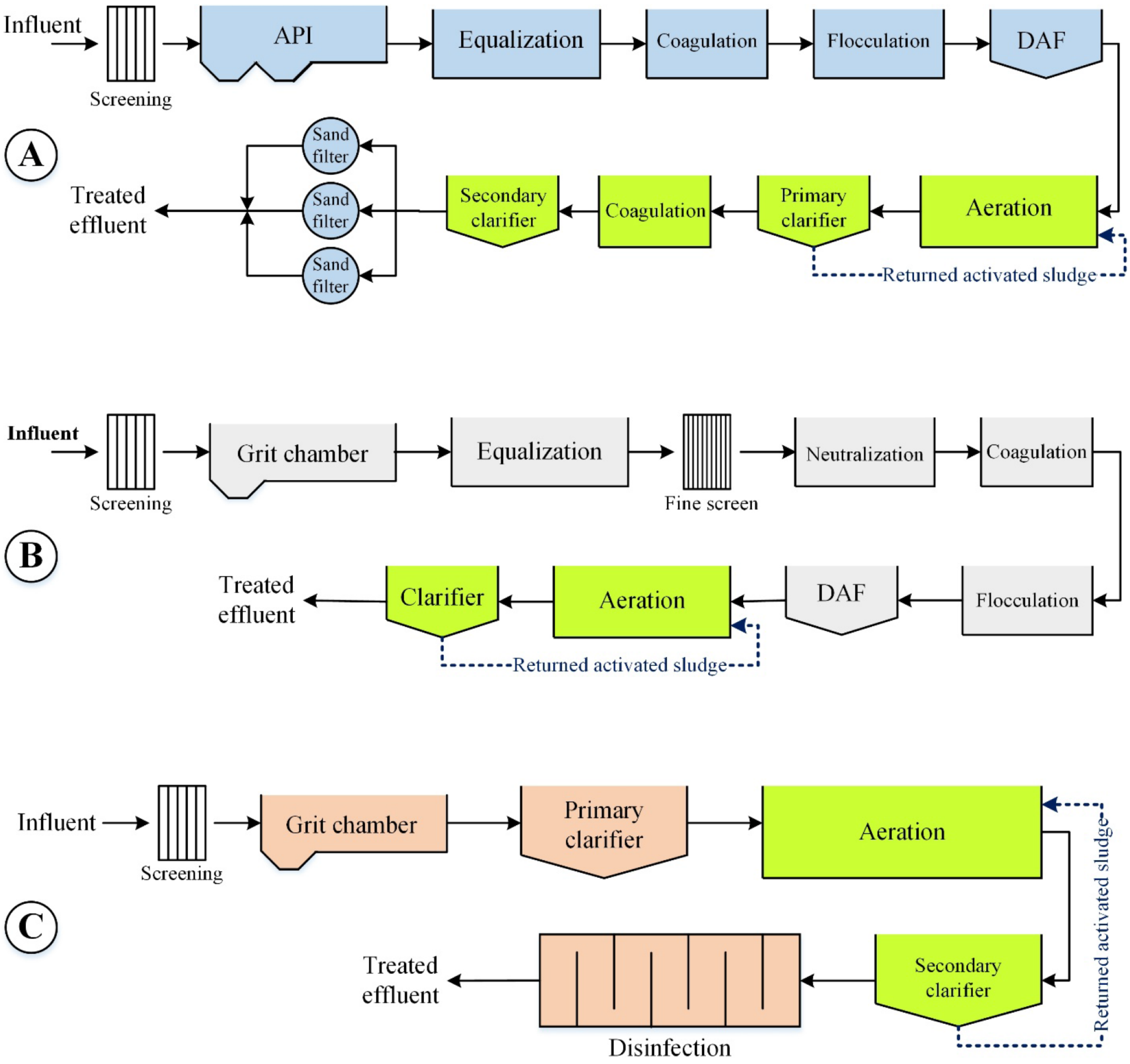

Fig. 1. Flow diagrams of the studied WWTPs: (A) PWWTP; (B) ABS-WWTP; and (C) MWWTP.

macro and micro-examination of morphological characters [27]. Bacteria were cultured in the R2A medium at 30°C and counted after 2–7 d of incubation [28].

### 2.5. Determination of fungal diversity

The fungal community diversity in each WWTPs was analyzed using Shannon diversity index (*H*), by the following equation [29]:

$$H = -\sum_{i=1}^{S} \left( \frac{n_i}{N} \right) \left( \log_2 \frac{n_i}{N} \right)$$

where *H* is the index of species diversity, *S* is the number of species encountered, $n_i/N$ is the proportion of the entire population that is made up by species *i*, and $\sum$ is the sum from species 1 to species *S*.

## 3. Results and discussion

### 3.1. Characteristics of WWTPs

Statistical analysis of data obtained from the measurements is presented in Table 2. Unlike the MWWTP, high standard deviations in the influent parameters of PWWTP and ABS-WWTP indicated the high variability of both industrial waste waters. On the other hand, a look at the $BOD_5$/COD ratio in the influents (0.73, 0.58 and 1.6 for PWWTP, ABS-WWTP and MWWTP, respectively) indicates higher biodegradability of municipal wastewater than both industrial ones [30]. Taking into account the influent COD values in WWTPs, both petrochemical and ABS resin manufacturing wastewater are classified as medium polluted industrial waste waters [31], and similarly, the municipal wastewater is classified as medium polluted municipal waste water [30].

Table 2
Statistics of the influent and effluent data

| | N | PWWTP | ABS-WWTP | MWWTP |
|---|---|---|---|---|
| Influent | | | | |
| COD (mg/L) | 23 | 1320 ± 230 (1060, 1558) | 1345 ± 415 (900, 1720) | 500 ± 26 (470, 540) |
| BOD$_5$ (mg/L) | 23 | 967 ± 491 (460, 1440) | 783 ± 389 (430, 1200) | 308 ± 23 (260, 340) |
| pH | 23 | 7.98 ± 1.45 (6.59, 10.03) | 6.37 ± 0.16 (6.19, 6.47) | 8.08 ± 0.08 (7.93, 8.25) |
| Temperature (°C) | 23 | 33.6 ± 1.1 (31.6, 35.0) | 34.3 ± 0.8 (33.6, 35.4) | 22.9 ± 2.3 (18.8, 25.5) |
| TSS (mg/L) | 22 | 227 ± 174 (72, 477) | 637 ± 147 (520, 850) | 323 ± 25.5 (295, 390) |
| NH$_3$-N (mg/L) | 23 | 9.1 ± 6.9 (1.7, 15.4) | 52.2 ± 25.6 (29.5, 79.4) | 47.8 ± 2.6 (44.5, 51.0) |
| NO$_3^-$ (mg/L) | 23 | 4.26 ± 0.87 (3.40, 5.45) | 89.3 ± 32.0 (44.0, 115.2) | 2.92 ± 0.44 (2.53, 3.53) |
| PO$_4^{3-}$ (mg/L) | 23 | 10.1 ± 6.6 (2.3, 18.3) | 25.4 ± 17.7 (0.8, 46.0) | 19.3 ± 0.7 (18.2, 20.1) |
| Oil (mg/L) | 14 | 41.5 ± 8.2 (34.0, 53.0) | – | 24.2 ± 2.5 (20.0, 26.0) |
| Phenol (mg/L) | 14 | 5.9 ± 2.2 (2.6, 7.2) | – | – |
| CN$^-$ (μg/L) | 14 | 3.78 ± 1.98 (1.30, 6.10) | 12.28 ± 1.96 (9.80, 14.60) | – |
| Effluent | | | | |
| COD (mg/L) | 23 | 100 ± 13 (88, 118) | 260 ± 46 (220, 310) | 39.5 ± 7.0 (30, 52) |
| BOD$_5$ (mg/l) | 23 | 54 ± 23 (37, 81) | 55.7 ± 32.9 (20.1, 85.3) | 25.2 ± 4.3 (20, 35) |
| pH | 23 | 7.67 ± 0.76 (6.70, 8.48) | 7.06 ± 0.55 (6.43, 7.38) | 7.40 ± 0.13 (7.25, 7.69) |
| Temperature (°C) | 23 | 27.9 ± 0.8 (26.4, 29.5) | 28.8 ± 0.9 (27.9, 29.9) | 23.2 ± 2.5 (19, 26) |
| TSS (mg/L) | 22 | 4 ±1 (3, 5) | 282 ± 151 (180, 456) | 25.8 ± 3.7 (22, 33) |
| NH$_3$-N (mg/L) | 23 | 0.8 ± 0.5 (0.1, 1.2) | 32.6 ± 21.5 (1.7, 74.5) | 2.0 ± 0.6 (1.4, 2.8) |
| NO$_3^-$ (mg/L) | 23 | 10.1 ± 2.9 (7.2, 13.6) | 218 ± 120 (118, 388) | 15.3 ± 0.7 (14.4, 16.0) |
| PO$_4^{3-}$ (mg/L) | 23 | 3.7 ± 3.1 (0.9, 8.0) | 11.2 ± 8.8 (1.1, 25.6) | 10.8 ± 0.7 (9.8, 11.7) |
| Oil (mg/L) | 14 | 12.0 ± 4.9 (8.0, 19.0) | – | 4.1 ± 2.3 (2.0, 7.3) |
| Phenol (mg/L) | 14 | 0.01 ± 0.01 (0.01, 0.02) | – | – |
| CN$^-$ (μg/L) | 14 | 1.00 ± 0.71 (0.10, 1.80) | 3.57 ± 1.27 (2.60, 5.00) | – |
| Bioreactor influent | | | | |
| COD (mg/L) | 12 | 415 ± 134 (280, 580) | 639 ± 256 (350, 840) | 305 ± 11 (286, 330) |
| BOD$_5$ (mg/L) | 12 | 175 ± 91 (70, 231) | 237 ± 47 (200, 290) | 191 ± 10 (175, 210) |
| Phenol (mg/L) | 12 | 4.87 ± 1.68 (2.60, 6.57) | – | – |
| CN$^-$ (μg/L) | 12 | – | 5.77 ± 0.76 (4.90, 6.30) | – |
| Bioreactor effluent | | | | |
| COD (mg/L) | 12 | 260 ± 67 (193, 350) | 283 ± 54 (211, 351) | 61.2 ± 7.6 (49.0, 77.0) |
| BOD$_5$ (mg/L) | 12 | 94 ± 53 (60, 155) | 86 ± 41 (30.6, 102.0) | 36.2 ± 8.1 (27.1, 51.2) |
| pH | 12 | 7.36 ± 0.46 (6.77, 7.81) | 7.23 ± 0.61 (6.5, 7.83) | 7.51 ± 0.17 (7.25, 7.77) |
| NH$_3$-N (mg/L) | 12 | 1.31 ± 0.35 (1.01, 1.74) | 51.5 ± 46.5 (0.9, 92.5) | 2.0 ± 0.6 (1.4, 2.8) |
| NO$_3^-$(mg/L) | 12 | 12.3 ± 5.3 (6.7, 18.1) | 176 ± 131 (122, 301) | 15.3 ± 0.7 (14.4, 16.0) |
| PO$_4^{3-}$ (mg/L) | 12 | 4.79 ± 3.58 (1.10, 9.60) | 17.36 ± 16.17 (0.87, 33.20) | 10.8 ± 0.7 (9.8, 11.7) |
| Phenol (mg/L) | 12 | 0.02 ± 0.01 (0.01, 0.03) | – | – |
| CN$^-$ (μg/L) | 12 | – | 4.61 ± 1.15 (3.25, 5.45) | – |

Data are expressed as mean ± S.D. (min, max).

### *3.2. Fungal abundance in WWTPs*

The total number of microorganisms enumerated by counting colony-forming units (cfu) is presented in Table 3. The results indicated that the total number of cultivable bacteria and fungi were in the ranges of $10^7$ to $10^8$ and $10^3$ to $10^4$ per mL of activated sludge, respectively, which were in compliance with the most enumeration results of bacteria and fungi in typical activated sludge systems [32]. Moreover, the highest values of bacteria and fungi were observed in MWWTP, and the total numbers of both bacteria and fungi were slightly similar in PWWTP and ABS-WWTP. In all plants, bacteria were the dominant population and the ratio of fungi to bacteria was significantly low, especially in the case of ABS-WWTP.

No significant difference was found in the total number of fungi counted on SDA and RBA media (data not shown). Shokrollahzadeh et al. [1], reported the total number of fungi

Table 3
Microbial concentrations in the activated sludge

| Activated sludge sample | Bacteria plate count (cfu/mL) | Fungi plate count (cfu/mL) | Fungi to bacteria plate count ratio |
|---|---|---|---|
| PWWTP | $(1.20 \pm 0.48) \times 10^7$ | $(3.60 \pm 0.52) \times 10^3$ | $1 : 3 \times 10^3$ |
| ABS-WWTP | $(1.90 \pm 0.26) \times 10^7$ | $(2.40 \pm 0.30) \times 10^3$ | $1 : 8 \times 10^3$ |
| MWWTP | $(5.30 \pm 0.77) \times 10^8$ | $(1.10 \pm 0.37) \times 10^4$ | $1 : 5 \times 10^4$ |

Table 4
Operational parameters of biological units

| Parameter | Unit | Measured data | | | Design criteria [30] |
|---|---|---|---|---|---|
| | | PWWTP | ABS-WWTP | MWWTP | |
| DO | mg/L | 3.9 ± 0.4 | 3.1 ± 0.5 | 1.7 ± 0.1 | |
| pH | – | 6.5 ± 1.4 | 7.1 ± 0.1 | 7.5 ± 0.2 | |
| Temperature | °C | 30.3 ± 0.9 | 31.2 ± 2.8 | 23.0 ± 2.3 | |
| HRT | h | 20.6 | 19.4 | 4.0–6.0 | 20–30 |
| SRT* | d | 39.3 | 21.2 | 8.0 | 20–40 |
| SVI | mL/g | 127.0 ± 11.0 | 276.5 ± 42.0 | 99.6 ± 17.7 | |
| MLSS | mg/L | 1463 ± 203 | 1816 ± 236 | 2012 ± 284 | 2000–5000 |
| MLVSS | mg/L | 1294 ± 175 | 1408 ± 191 | 1663 ± 288 | |
| F/M** | kg BOD/kg MLVSS.d | 0.14 | 0.15 | 0.12 | 0.04–0.10 |
| OLR*** | kg BOD/$m^3$.d | 0.1 | 0.2 | | 0.1–0.3 |
| RAS**** | % of influent | 98.5 | 99.1 | 75.0 | 50–150 |

in the biological reactor treating petrochemical wastewater in the range of $10^2$–$10^4$ cfu/mg, and the maximum number of aerobic heterotrophic bacteria about $10^8$ cfu/mg. In another study, Yang et al. [10], described the abundance and diversity of yeasts and other microorganisms in WWTPs. They found that the fungi to bacteria ratios in biopharmaceutical, municipal and paper making WWTPs were 1:9 $10^3$ to 1:6 $10^5$, 1:8 $10^2$ to 1:5 $10^3$ and 1:1 $10^3$, respectively.

It has been proved that the type of wastewater, operational parameters (such as pH, DO, temperature), and the specific environmental conditions exert more influence on the composition, diversity, and abundance of microbial communities in biological wastewater treatment systems [10]. Table 4 summarizes the operational parameters of the studied biological reactors. In general, the fungi prefer low pH values, aerobic conditions, and temperature of 25–30°C [33]. As shown in this table, DO, pH and temperature values in activated sludge reactors are favorable for growth and sporulation of fungal and yeast communities. Taking into account the operational data, all the WWTPs are worked in the absolute aerobic conditions (1.7–3.9 mg/L) with the temperature and pH contents within the optimal ranges for fungal growth (23–30°C and 6.5–7.5, respectively). However, the high fungi to bacteria ratio (1:3 $10^3$) were observed in PWWTP. Considering DO and pH values, the activated sludge of PWWTP is the most favorable environment for fungal growth compared to the others. DO is one of the most important parameters which can strongly affect the growth and sporulation of fungal and yeast communities. Yang et al. [10], found that the number of yeasts and the ratio of yeasts to total microorganisms in semi-aerobic reactors were higher than anaerobic reactors. As well, like other microorganisms, fungi and yeasts are profoundly affected by the pH value. At pH > 9.0, microbial activity is inhibited whereas at pH < 6.5, fungi dominate over the bacteria in the competition for the substrate [16]. Blagodatskaya and Anderson [34], proved that fungi to bacteria ratio increased as the pH value decreased.

Overall, the above-mentioned parameters might not have considerable influence on the abundance and diversity of fungal communities among the WWTPs because of the negligible difference in their values. So, the type of wastewater seems to be the most important factor which can strongly affect the diversity and abundance of fungal communities within the treatment systems. Comparing the treatment plants, the PWWTP receives a high variety of inlet streams (e.g. oily wastewater, saline wastewater, rainwater, etc.) comprising various types of recalcitrant organic compounds which can affect the abundance of the microbial population. The ABS resin wastewater was usually characterized by high concentrations of COD, total Kjeldahl nitrogen (TKN), total nitrogen (TN) and total phosphorus (TP). In addition, the presence of toxic and refractory compounds such as aromatics may inhibit growth and sporulation of some fungal species and form the specific fungal community structure [35,36]. Finally, the MWWTP receives a high volume of domestic wastewater rich in organic materials and nutrients compared to PWWTP and ABS-WWTP which provides favorable conditions for microorganisms (bacteria, fungi, yeast, etc.) as indicated by the high number of bacteria and fungi (Table 3).

### *3.3. Characterization of fungal species*

The diversity of fungal species in WWTPs was presented in Table 5. Based on the culture-dependent method, a total of 256 strains were isolated, purified and identified. All isolated strains were identified based on their growth, color and morphological characters. The strains have belonged to nine genera of *Penicillium*, *Chrysosporium*, *Aspergillus*, *Trichoderma*, *Trichothecium*, *Cladosporium*, *Fusarium*, *Geotrichum*, and *Acremonium*. The results of the comparative analysis showed that the most common fungal species belonged to genus *Geotrichum*, *Cladosporium*, *Aspergillus*, and *Penicillium*. All isolated fungi were filamentous (non-filamentous fungi were not detected) in the activated sludge samples. The increase in the number of filamentous organisms (bacteria and fungi) in the activated sludge systems can influence the settle ability and compatibility of the sludge, and subsequently affect the SVI. In general, SVI is used to determine the settling ability of the activated sludge. A sludge with the SVI < 80 has good settling properties while the high SVI (> 150) indicates settling problems and possibly bulking [16]. According to the Table 4, all the studied samples have SVI values higher than the optimum value of 80 (especially in the case of ABS-WWTP), which can partly be attributed to the presence of filamentous organisms. It has previously been proved that the SVI increases when the number of filamentous microorganisms increase in the biological reactor [37,38].

As mentioned before, the type of wastewater and operating conditions over the biological reactor can greatly influence the fungal distribution. Compared to the ABS-WWTP and MWWTP, the PWWTP receives various high concentrated waste streams which may promote the growth and sporulation of fungi and yeasts. According to Table 5, the highest diversity of fungal species was detected in PWWTP as indicated by the high Shannon diversity index ($H = 2.56$). A total of 85 and 3 fungal and yeast strains, respectively, belonging to 10 genera were isolated from the PWWTP. *Geotrichum sp.* was the most abundant genus in PWWTP and constituting 43.2% of all detected genera, followed by *Chrysosporium sp.* (17%), *Cladosporium sp.* (11.4%) and *Trichoderma sp.* (6.82%). These isolated strains represented about 78.4% of the total cultivable population of fungi in the activated sludge. In other studies, *Geotrichum sp.* and *Trichoderma sp.* were isolated from the activated sludge treating petrochemical wastewater [1,39].

The ABS-WWTP receives difficult wastewater stream containing many recalcitrant and toxic substances such as acrylonitrile, styrene, cyanide, etc., which form a specialized environment for the microorganisms to grow. It is clear from Table 5 that the fungal diversity was quite low in the activated sludge samples of ABS-WWTP compared to the PWWTP, as the Shannon index was 1.57. In ABS-WWTP, the population of *Aspergillus sp.* was predominant (63% of the total isolates), followed by *Cladosporium sp.* (21%) and *Trichoderma sp.* (8.8%). These groups occupied 95% of the total isolated fungal communities in ABS-WWTP. The genera of *Penicillium* and *Chrysosporium* were in low frequency in the ABS-WWTP (both were 3.5%). To our knowledge, no study has been found regarding the isolation of these strains from the ABS-WWTPs, however, degradation of styrene and cyanide (as important compounds present in the ABS wastewater) by different strains of *Aspergillus* have been documented in the previous studies [40–42].

A total of 113 fungal species representing 4 genera were collected from the activated sludge samples of the MWWTP (Table 5). The MWWTP showed the lowest fungal diversity among the studied WWTPs but the Shannon index calculated for this plant ($H = 1.95$) was relatively higher than that of the ABS-WWTP, which is because of the higher fungal strains present in MWWTP compared to ABS-WWTP. *Geotrichum sp.* was found to be the most common occurrence genus of samples (36.3% of the total count of fungi) in MWWTP. *Aspergillus sp.* occupied the second place in the case of the number of isolation matching 23.9% of total fungi. *Cladosporium sp.* and *Penicillium sp.* occupied the third and fourth position constituting 21.2 and 18.6% of the total isolates, respectively. These results were fully in line with the results reported in the previous studies [43–46]. Evans and Seviour [35], investigated the biodiversity of fungal communities in samples collected from the municipal and industrial WWTPs using a culture-independent method. They found the considerable differences of fungal biodiversity in the treatment plants. This may be explained mainly

Table 5
Diversity structure of fungal species

| Fungus | PWWTP | | ABS-WWTP | | MWWTP | |
|---|---|---|---|---|---|---|
| | No. of isolates | % of total isolates | No. of isolates | % of total isolates | No. of isolates | % of total isolates |
| *Penicillium* sp. | 5 | 5.7 | 2 | 3.5 | 21 | 18.6 |
| *Chrysosporium* sp. | 15 | 17 | 2 | 3.5 | – | – |
| *Aspergillus* sp. | 6 | 6.8 | 36 | 63.2 | 27 | 23.9 |
| *Trichoderma* sp. | 6 | 6.8 | 6 | 8.8 | – | – |
| *Trichothecium* sp. | 2 | 2.3 | – | – | – | – |
| *Cladosporium* sp. | 10 | 11.4 | 12 | 21.1 | 24 | 21.2 |
| *Fusarium* spp. | 1 | 1.1 | – | – | – | – |
| *Acremonium* sp. | 2 | 2.3 | – | – | – | – |
| *Geotrichum* sp. | 38 | 43.2 | – | – | 41 | 36.3 |
| Total | 85 | 100 | 58 | 100 | 113 | 100 |

due to the differences in the composition of waste waters which form a special fungal community structure [47].

In general, the isolated fungi from WWTPs were *Geotrichum*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Chrysosporium*, and *Trichoderma*. Among them, some species have been previously isolated from various wastewater treatment systems [43,46,48–50]. *Geotrichum sp.* was the most abundant and the most commonly distributed fungi (approximately > 40%) in both PWWTP and MWWTP however, it was not isolated from the activated sludge samples of ABS-WWTP. *Geotrichum sp.* is a ubiquitous filamentous yeast-like fungus found worldwide in the soil, water, and air samples. It was frequently detected as the most abundant fungal species in many activated sludge systems [45]. *Geotrichum sp.* were noted to be the effective degraders of petroleum compounds [51]. During the warm period (25°C), *Geotrichum* has a short doubling time (7 to 8 hours) to compete with *zoogleal* bacteria with a doubling time of 10 to 25 h so, its growth is intensified at high concentrations of $BOD_5$ (> 280 mg/L) [16].

*Aspergillus sp.* was observed in high frequency in the ABS-WWTP and occupied the fourth and second positions in the petrochemical and municipal WWTPs, respectively. *Aspergillus sp.* is tolerant to high temperature while, pH values in the range of 4 to 8 have little effect on its growth [52,53]. Therefore, the studied petrochemical and ABS waste waters are favorable environments for growth and spore germination of *Aspergillus sp.* due to the relatively high temperature (~31°C) and optimal pH value (6–7). Fungi belonging to these genera have been found to be responsible for degrading a wide range of contaminants in the petrochemical and petroleum effluents. To date, degradation of the starch-graft-acrylonitrile copolymer, phenol, refinery effluent and petroleum hydrocarbons by *Aspergillus Niger* [17,24,54], aliphatic and aromatic hydrocarbons by *Aspergillus terreus* [55], and phenol by immobilized *Aspergillus fumigatus* in batch and packed bed reactors [23] have been documented in the ligterature.

*Cladosporium sp.* was detected in moderate frequency at all WWTPs, with recoveries of 21% from the both ABS-WWTP and MWWTP as well as 11.4% from PWWTP. *Cladosporium* species are ubiquitous worldwide and commonly isolated from the soil and organic matters. Salvo et al. [56], reported *Cladosporium* as an abundant genus in sediments impacted by PAHs. In another study, Li et al. [57], reported the great ability of genus *Cladosporium* in degradation of diesel oil with a real reduction ratio of up to 34% after 5 days. *Penicillium sp.* was isolated in low frequency from the PWWTP and ABS-WWTP and in moderate frequency from the MWWTP. In fact, the cool and moderate climates are desirable for *Penicillium* species and they grow very well at 18 to 25°C, whereas, most bacteria grow better at a higher temperature of 30 to 38°C. Kacprzak et al. [44], found that *Penicillium* species occupied about 50% of all existing fungal communities in wastewater sludge. The genus *Chrysosporium* was the second most common fungi isolated from the PWWTP. Various species of *Chrysosporium* have been formerly isolated from the wastewater and activated sludge samples [45,58]. *Trichoderma sp.* was observed in low frequency and comprised about 7 and 9% of all fungal communities isolated from PWWTP and ABS-WWTP, respectively. The distribution of *Trichoderma* species in the wastewater and activated sludge samples have been reported in many related studies [43,49,59] however, this genus was not detected in the MWWTP sludge samples. Verma et al. [60], found that *Trichoderma* species were capable of producing laccase enzyme, with the potential for degradation of toxic organic compounds. *Acremonium*, *Trichothecium*, and *Fusarium* were isolated in rare frequencies from the PWWTP and they were not detected in any activated sludge samples collected from the both MWWTP and ABS-WWTP.

In summary, as compared with bacteria, fungi are rarely found as dominant groups of microorganisms in the activated sludge systems. However, in some specific environments such as toxic or highly acidic conditions, cold temperatures, and nitrogen-deficient conditions, fungi grow well in competition with bacteria [35,61]. Moreover, in some hazardous waste waters such as hospital waste waters containing high concentrations of antibiotics which can affect the bacterial activity, fungal treatment is confirmed as a promising technology considering the high degradation potential of fungi against hazardous and recalcitrant compounds [62]. Therefore, further studies on the capabilities of isolated fungal communities in degradation and detoxification of hazardous pollutants will be helpful to deepen scientific understanding of the biological treatment of difficult wastewaters.

## 4. Conclusions

In this research, the abundant and diverse fungal communities were studied in three full-scale activated sludge systems. The results showed considerable differences in fungal biodiversity of the treatment plants which may be due to the existence of a wide variety of compounds in the influents and also operational parameters of the plants. The members of the fungal communities were assigned to 256 strains and nine genera. Among them, 85 strains were isolated from the PWWTP, 58 strains from the ABS-WWTP and 113 strains from the MWWTP samples. The most-frequently isolated fungi from the studied WWTPs have belonged to the genera *Geotrichum*, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Trichoderma*.

This study, for the first time, evaluated the fungal population diversity and structure in a petrochemical and an ABS resin manufacturing WWTP using the culture-dependent method. The findings will surely enhance our understanding in relation to the fungal communities existing in the activated sludge. As with bacteria, fungi and yeasts are eco-friendly agents and can naturally develop modified metabolism to deal with the environmental contaminants. Therefore, extensive studies are needed to investigate fungal species and their functions in the biological wastewater treatment systems.

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## References

[1] S. Shokrollahzadeh, F. Azizmohseni, F. Golmohammad, H. Shokouhi, F. Khademhaghighat, Biodegradation potential and bacterial diversity of a petrochemical wastewater treatment plant in Iran, Bioresour. Technol., 99 (2008) 6127–6133.

[2] K. Hashimoto, M. Matsuda, D. Inoue, M. Ike, Bacterial community dynamics in a full-scale municipal wastewater treatment plant employing conventional activated sludge process, J. Biosci. Bioeng., 118 (2014) 64–71.

[3] X. Wang, Y. Xia, X. Wen, Y. Yang, J. Zhou, Microbial community functional structures in wastewater treatment plants as characterized by geochip, PLoS One., 9 (2014) e93422.

[4] V.M.F. Alexandre, T.M.S. de Castro, L.V. de Araújo, V.M.J. Santiago, D.M.G. Freire, M.C. Cammarota, Minimizing solid wastes in an activated sludge system treating oil refinery wastewater, Chem. Eng. Process, 103 (2016) 53–62.

[5] A. Behnami, M. Shakerkhatibi, R. Dehghanzadeh, K.Z. Benis, S. Derafshi, E. Fatehifar, The implementation of data reconciliation for evaluating a full-scale petrochemical wastewater treatment plant, Environ. Sci. Pollut. Res., 23 (2016) 22586–22595.

[6] K.Z. Benis, M. Shakerkhatibi, R. Yousefi, D. Kahforoushan, S. Derafshi, Emission patterns of acrylonitrile and styrene around an industrial wastewater treatment plant in Iran, Int. J. Environ. Sci. Technol., 13 (2016) 2353–2362.

[7] K. Obahiagbon, E. Agbonghae, N. Amenaghawon, Effect of microbial load of Aspergillus niger and Pseudomonas aeruginosa on the bioremediation of crude oil polluted water, J. Mater. Environ. Sci., 5 (2014) 1786–1791.

[8] Y. Chao, Y. Mao, K. Yu, T. Zhang, Novel nitrifiers and comammox in a full-scale hybrid biofilm and activated sludge reactor revealed by metagenomic approach, Appl. Microbiol. Biotechnol., 100 (2016) 8225–8237.

[9] H. Yahong, Q. Shan, M. Fang, W. Jue, Short-Term and long-term effects of tourmaline on activated sludge viability and performance, Water Air Soil Pollut., 228 (2017) 395.

[10] Q. Yang, F.E. Angly, Z. Wang, H. Zhang, Wastewater treatment systems harbor specific and diverse yeast communities, Biochem. Eng. J., 58–59 (2011) 168–176.

[11] B. Song, G. Zeng, J. Gong, J. Liang, P. Xu, Z. Liu, Y. Zhang, C. Zhang, M. Cheng, Y. Liu, S. Ye, H. Yi, X. Ren, Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals, Environ. Int., 105 (2017) 43–55.

[12] A. Behnami, D. Farajzadeh, S. Isazadeh, K.Z. Benis, M. Shakerkhatibi, Z. Shiri, S. Ghorghanlu, A. Yadeghari, Diversity of bacteria in a full-scale petrochemical wastewater treatment plant experiencing stable hydrocarbon removal, J. Water Process Eng., 23 (2018) 285–291.

[13] R. Chandra, R.N. Bharagava, A. Kapley, H.J. Purohit, Bacterial diversity, organic pollutants and their metabolites in two aeration lagoons of common effluent treatment plant (CETP) during the degradation and detoxification of tannery wastewater, Bioresour. Technol., 102 (2011) 2333–2341.

[14] N.P. Cheremisinoff, Biotechnology for waste and wastewater treatment, Noyes Publications, Fairview Avenue, Westwood, New Jersey 07675, United States, 1996.

[15] C. Cortés-Lorenzo, A. González-Martínez, H. Smidt, J. González-López, B. Rodelas, Influence of salinity on fungal communities in a submerged fixed bed bioreactor for wastewater treatment, Chem. Eng. J., 285 (2016) 562–572.

[16] N.F. Gray, Biology of wastewater treatment, 2nd ed., Imperial College Press, United Kingdom, 2004.

[17] L. Tebbouche, D. Hank, S. Zeboudj, A. Namane, A. Hellal, Evaluation of the phenol biodegradation by Aspergillus niger: application of full factorial design methodology, Desal. Water Treat., 57 (2015) 6124–6130.

[18] L. Niu, Y. Li, L. Xu, P. Wang, W. Zhang, C. Wang, W. Cai, L. Wang, Ignored fungal community in activated sludge wastewater treatment plants: diversity and altitudinal characteristics, Environ. Sci. Pollut. Res., (2016) 1–9.

[19] V.L. Santos, V.R. Linardi, Biodegradation of phenol by a filamentous fungi isolated from industrial effluents-identification and degradation potential, Process Biochem., 39 (2004) 1001–1006.

[20] S.D. Weber, A. Hofmann, M. Pilhofer, G. Wanner, R. Agerer, W. Ludwig, K.H. Schleifer, J. Fried, The diversity of fungi in aerobic sewage granules assessed by 18S rRNA gene and ITS sequence analyses, FEMS Microbiol. Ecol., 68 (2009) 246–254.

[21] A.R. MacGillivray, M.P. Shiaris, Biotransformation of polycyclic aromatic hydrocarbons by yeasts isolated from coastal sediments, Appl. Environ. Microbiol., 59 (1993) 1613–1618.

[22] F. Chaillan, A. Le Flèche, E. Bury, Y.h. Phantavong, P. Grimont, A. Saliot, J. Oudot, Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms, Res. Microbiol., 155 (2004) 587–595.

[23] P. Balamurugan, B. Preetha, T. Virithagiri, Study on effect of operating parameters on biodegradation of phenol by Aspergillus Fumigatus, Int. J. Eng. Res. Appl., 2 (2012) 981–986.

[24] G. Sabah, E. Jatau, C. Whong, Assessment of biodegradation ability of aspergillus niger isolated from mechanic workshops soilon refinery effluent and petroleum hdrocarbons, Int. J. Sci. Res. Pub., 6 (2016) 381–389.

[25] R. Bouchiat, E. Veignie, D. Grizard, C. Soebert, M. Vigier, C. Rafin, Ability of filamentous fungi to degrade four emergent water priority pollutants, Desal. Water Treat., 57 (2016) 6740–6746.

[26] APHA, Standard methods for the examination of water and wastewater, 21st ed., American Public Health Association, Washington, United State, 2005.

[27] J. Oudot, J. Dupont, S. Haloui, M.F. Roquebert, Biodegradation potential of hydrocarbon-assimilating tropical fungi, Soil Biol. Biochem., 25 (1993) 1167–1173.

[28] R. Margesin, G.A. Płaza, S. Kasenbacher, Characterization of bacterial communities at heavy-metal-contaminated sites, Chemosphere, 82 (2011) 1583–1588.

[29] C.E. Shannon, A mathematical theory of communication, Bell Syst. Tech. J., 27 (1948) 623–656.

[30] G. Tchobanoglous, F. Burton, H. Stensel, Wastewater engineering: treatment and reuse, fourth ed., McGraw-Hill Higher Education, New York, United State, 2003.

[31] L. Dao, T. Grigoryeva, A. Laikov, R. Devjatijarov, O. Ilinskaya, Full-scale bioreactor pretreatment of highly toxic wastewater from styrene and propylene oxide production, Ecotoxicol. Environ. Saf., 108 (2014) 195–202.

[32] G. Bitton, Wastewater microbiology, 3rd ed., John Wiley & Sons, 2005.

[33] M. Tuomela, M. Vikman, A. Hatakka, M. Itävaara, Biodegradation of lignin in a compost environment: a review, Bioresour. Technol., 72 (2000) 169–183.

[34] E.V. Blagodatskaya, T.-H. Anderson, Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and $qCO_2$ of microbial communities in forest soils, Soil Biol. Biochem., 30 (1998) 1269–1274.

[35] T.N. Evans, R.J. Seviour, Estimating biodiversity of fungi in activated sludge communities using culture-independent methods, Microb. Ecol., 63 (2012) 773–786.

[36] B. Lai, Y. Zhou, P. Yang, Treatment of wastewater from acrylonitrile–butadiene–styrene (ABS) resin manufacturing by Fe0/GAC–ABFB, Chem. Eng. J., 200–202 (2012) 10–17.

[37] D.H. Eikelboom, Process control of activated sludge plants by microscopic investigation, IWA publishing, 2000.

[38] M.X. Li, A.J. Li, Q. Sun, X.M. Jiang, S.H. Chen, Enhancement of biodiesel production by cultivating Dipodascaceae moderated-filamentous granular sludge with sugar-containing wastewater, Int. Biodeterior. Biodegrad., 110 (2016) 38–45.

[39] H. Dave, C. Ramakrishna, J.D. Desai, Degradation of acrylic acid by fungi from petrochemical activated sludge, Biotechnol. Lett., 18 (1996) 963–964.

[40] J. Paca, B. Koutsky, M. Maryska, M. Halecky, Styrene degradation along the bed height of perlite biofilter, J. Chem. Technol. Biotechnol., 76 (2001) 873–878.

[41] S. Das, S. Santra, Cyanide degradation by Aspergillus niger strain isolated from steel-plant wastewater, EJEAFChe 10 (2011)

[42] L. Sabatini, C. Ferrini, M. Micheloni, A. Pianetti, B. Citterio, C. Parlani, F. Bruscolini, Isolation of a strain of Aspergillus fumigatus able to grow in minimal medium added with an industrial cyanide waste, World J. Microbiol. Biotechnol., 28 (2012) 165–173.

[43] A. Fakhrul-Razi, M.Z. Alam, A. Idris, S. Abd-Aziz, A.H. Molla, Filamentous fungi in Indah Water Konsortium (IWK) sewage treatment plant for biological treatment of domestic wastewater sludge, J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng., 37 (2002) 309–320.

[44] M. Kacprzak, E. Neczaj, E. Okoniewska, The comparative mycological analysis of wastewater and sewage sludges from selected wastewater treatment plants, Desalination, 185 (2005) 363–370.

[45] M.F. Awad, M. Kraume, Keratinophilic fungi in activated sludge of wastewater treatment plants with MBR in Berlin, Germany, Mycology, 2 (2011) 276–282.

[46] G. Helal, M. Mostafa, M. El-Said, Fungi in the sewage-treatment Zeinein plant, Cairo, Egypt, J. Basic Appl. Mycol. Egypt, 2 (2011) 69–82.

[47] T.N. Evans, G. Watson, G.N. Rees, R.J. Seviour, Comparing activated sludge fungal community population diversity using denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism, Antonie van Leeuwenhoek, 105 (2014) 559–569.

[48] W.B. Cooke, W.O. Pipes, The occurrence of fungi in activated sludge, Mycopathol. Mycol. Appl., 40 (1970) 249–270.

[49] T.T. More, S. Yan, R.D. Tyagi, R.Y. Surampalli, Potential use of filamentous fungi for wastewater sludge treatment, Bioresour. Technol., 101 (2010) 7691–7700.

[50] H. Varadarajan, S. Shikha, Biodiversity characterization of bacterial and fungal isolates from gold electroplating industry effluents, J. Appl. Environ. Microbiol., 2 (2014) 212–219.

[51] E. Bogusławska-Wąs, W. Dąbrowski, The seasonal variability of yeasts and yeast-like organisms in water and bottom sediment of the Szczecin Lagoon, Int. J. Hyg. Environ. Health, 203 (2001) 451–458.

[52] N. Magan, J. Lacey, Effect of water activity, temperature and substrate on interactions between field and storage fungi, Trans. Brit. Mycol. Soc., 82 (1984) 83–93.

[53] M.A. Gock, A.D. Hocking, J.I. Pitt, P.G. Poulos, Influence of temperature, water activity and pH on growth of some xerophilic fungi, Int. J. Food Microbiol., 81 (2003) 11–19.

[54] A.H. Navarchian, A. Sharafi, R.K. Kermanshahi, Biodegradation Study of Starch-graft-Acrylonitrile Copolymer, J. Polym. Environ., 21 (2013) 233–244.

[55] J.C. Colombo, M. Cabello, A.M. Arambarri, Biodegradation of aliphatic and aromatic hydrocarbons by natural soil microflora and pure cultures of imperfect and lignolitic fungi, Environ. Pollut., 94 (1996) 355–362.

[56] V.S. Salvo, I. Gallizia, M. Moreno, M. Fabiano, Fungal communities in PAH-impacted sediments of Genoa-Voltri Harbour (NW Mediterranean, Italy), Marine Poll. Bull., 50 (2005) 553–559.

[57] Y.Q. Li, H.F. Liu, Z.L. Tian, L.H. Zhu, Y.H. Wu, H.Q. Tang, Diesel pollution biodegradation: synergetic effect of Mycobacterium and filamentous fungi, Biomed. Environ. Sci., 21 (2008) 181–187.

[58] A.I.I. Abdel-Hafez, H.M.M. El-Sharouny, The occurrence of keratinophilic fungi in sewage sludge from Egypt, J. Basic Microbiol., 30 (1990) 73–79.

[59] M.F. Awad, M. Kraume, The occurrence of fungi in activated sludge from MBRs, Proc. ICESE 2010: International Conference on Environmental Sciences and Engineering, 2010, pp. 540–543.

[60] M. Verma, S.K. Brar, R.D. Tyagi, R.Y. Surampalli, J.R. Valero, Industrial waste waters and dewatered sludge: rich nutrient source for production and formulation of biocontrol agent, Trichoderma viride, World J. Microbiol. Biotechnol., 23 (2007) 1695–1703.

[61] D. Smith, M.J. Ryan, J.G. Day, The UK national culture collection (UKNCC) Biological Resource: Properties, Maintenance and Management, United Kingdom national culture collection, 2001.

[62] D. Lucas, D. Barceló, S. Rodriguez-Mozaz, Removal of pharmaceuticals from wastewater by fungal treatment and reduction of hazard quotients, Sci. Total. Environ., 571 (2016) 909–915.