Science of the Total Environment 627 (2018) 835–843

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Tannery mixed liquors from an ecotoxicological and mycological point of view: Risks vs potential biodegradation application

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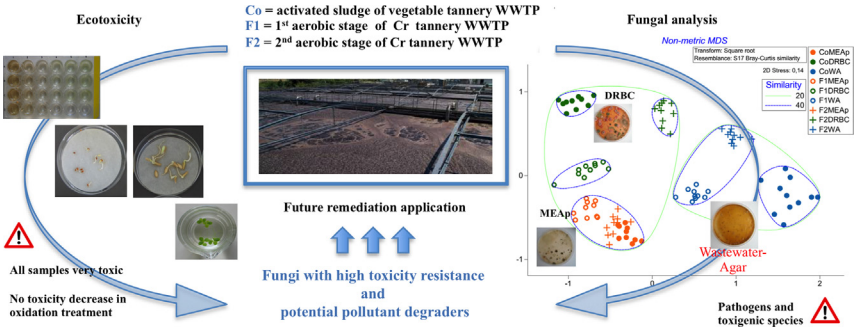
HIGHLIGHTS

- We identified 100 fungi (63 sample-specific) in activated sludge of tannery WWTPs.
- High ecotoxicity and health (pathogens and toxins) risks are associated to samples.
- Culture medium affected the mycoflora more than incubation temperature.
- Agarised wastewater as medium allowed identifying fungi with biodegradation potential.
- P. boydii complex was the most abundant on WA (role in degradation of pollutants?).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form 23 January 2018
Accepted 24 January 2018
Available online xxxx

Editor: Zhen (Jason) He

Keywords:
Tannery wastewater
Activated sludge
Fungi
Autochthonous mycoflora
Ecotoxicity

ABSTRACT

Fungi are known to be present in the activated sludge of wastewater treatment plants (WWTP). Their study should be at the base of an overall vision of the plant effectiveness and of effluents sanitary impact. Moreover, it could be fundamental for the implementation of successful bioaugmentation strategies aimed at the removal of recalcitrant or toxic compounds. This is one of the first studies on the cultivable autochthonous mycoflora present in the mixed liquors of two WWTP treating either vegetable or chromium tannery effluents. All samples showed a risk associated with potential pathogens or toxigenic species and high ecotoxicity (*Lepidium sativum* and *Raphidocelis subcapitata* were the most sensitive organisms). Diverse fungal populations developed, depending on the origin of the samples (63% of the 102 identified taxa were sample-specific). The use of a fungistatic was determinant for the isolation and, thus, for the identification of sample-specific species with a lower growth rate. The incubation temperature also affected the mycoflora composition, even though at lower extent. A selective medium, consisting of agarised wastewater, allowed isolating fungi with a biodegradation potential. *Pseudallescheria boydii/Scedosporium apiospermum* species complex was ubiquitously dominant, indicating a possible role in the degradation of pollutants in both WWTP. Other species, i.e. *Trichoderma spp.*, *Trematosphaeria grisea*, *Geotrichum candidum*, *Lichtheimia corymbifera*, *Acremonium furcatum*, *Penicillium simplicissimum*, *Penicillium dangeardii*, *Fusarium solani*, *Scopulariopsis brevicaulis* potentially could be involved in the degradation of specific pollutants of vegetable or chromium tannery wastewaters. However, several of these fungi are potential pathogens and their application, for an in situ treatment, must be carefully evaluated.

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https://doi.org/10.1016/j.scitotenv.2018.01.240
0048-9697/

1. Introduction

The secondary treatments, which consist of biological processes operated with either suspended or attached biomasses, are generally the core of wastewater treatment plants (WWTPs) (Leyva-Diaz et al., 2017). The knowledge on composition and dynamics of microbial community has been fundamental for the development of biological treatment technologies (Orhon, 2015). However, studies were mainly focused on the bacterial fraction of these complex ecosystems, as well as mathematical models applied to describe the processes (Orhon, 2015). Recently, the literature has pointed out a consistent presence of autochthonous mycoflora in the activated sludge of WWTPs treating paper mill, vinery or municipal wastewaters (Grabinskaloniewska et al., 1993; Awad and Kraume, 2011; Evans and Seviour, 2012). Its characterisation should be included in an overall vision of the effectiveness and impact of a treatment plant, considering functional, ecological and sanitary aspects (More et al., 2010; Awad and Kraume, 2011; Korzeniewska, 2011).

The role of fungal organisms in the depuration process has been recently demonstrated. In particular, fungi seem to perform a complementary action with respect to bacteria in the removal of pollutants from wastewaters and can actively interact with them (Liu et al., 2017; Svobodová et al., 2016; Anastasi et al., 2012).

The study of autochthonous fungi in WWTPs may acquire particular importance when recalcitrant or toxic pollutants are present in wastewaters, in order to improve depuration performance by bioaugmentation or biostimulation of naturally present fungi (More et al., 2010; Djelal and Amrane, 2013; Herrero and Stuckey, 2015).

Tannery wastewaters, regardless of the type of industrial process (chromium or vegetable), are among the most difficult to treat, basically on account of their recalcitrance and/or their toxicity towards bacteria (Lofrano et al., 2013). On the contrary, the ability of fungi in the degradation of tannery pollutants has been already demonstrated (Sharma and Malaviya, 2016; Zhang et al., 2015).

Besides the study of diversity and ecological function of the microbial community, a successful bioaugmentation strategy should contemplate the assessment of possible toxicity effects towards the biota (Herrero and Stuckey, 2015). Ecotoxicity tests, associated with chemical and biological characterisations, can be an informative tool for the efficiency evaluation and the management of the secondary treatment in WWTPs (Chapman, 2000). Nevertheless, these procedures are not so diffused at industrial level. This is mainly due to the lack of knowledge about the methods for data interpretation and to the complexity of the information that these analyses can provide (Chapman, 2000).

The present study is focused on the analysis of cultivable autochthonous mycobiota present in mixed liquors of the oxidation tanks of two wastewater treatment plants, which collect vegetable and chromium tannery effluents, respectively. Selective media were exploited for the isolation of fungi with biodegradation potential and/or the ability to compete with other microorganisms. Moreover, the samples were incubated at both 25 °C and 15 °C, in order to acquire information about the effect of seasonal temperature fluctuations on mycoflora development. Finally ecotoxicological aspects of the samples were assessed by means of four bioassays.

2. Materials and methods

2.1. Wastewaters

The samples (three in total) were collected from two WWTPs located in Tuscany (Italy):

Co – mixed liquor from the aerobic tanks of Cuoiodepur vegetal tannery WWTP (San Miniato, Pisa, Italy).

F1 – effluent from the settler of the first aerobic stage of the chromium tannery WWTP of Consorzio Aquarno SpA (Santa Croce sull'Arno, Pisa, Italy).

F2 - mixed liquor from the second biological stage of chromium tannery wastewater treatment plant of Consorzio Aquarno SpA (Santa Croce sull'Arno, Pisa, Italy).

Cuoiodepur manages a consortial WWTP, where the effluents of about 100 tanneries, operating vegetable tanning process, are treated. The influent wastewaters are characterised by a high organic and nitrogen load (COD > 15,000 mg L^{-1}), high salinity and by the presence of natural and synthetic tannins. The Cuoiodepur WWTP treats about 5,000 m^3 d^{-1} of tannery wastewaters and the treatment train is composed of: pretreatments, simultaneous equalization and sulphide oxidation with pure oxygen, primary settling, conventional suspended activated sludge (denitrification nitrification) biological stage and coagulation flocculation as tertiary treatment. The temperature of the biological reactors ranges between 19 °C and 35 °C, the hydraulic retention time (HRT) is about 3 days and the solids retention time (SRT) is usually between 50 and 70 d.

Aquarno is a joint-stock company by large majority private operating in the Tuscany tannery district for the treatment of chromium tannery wastewaters. The Aquarno WWTP treats about 12,000 m^3 d^{-1} of chromium tannery wastewaters. The treatment train is composed of: pretratments, aerobic activated sludge system (at low SRT to remove sulphide), second conventional activated sludge system for nitrogen and carbon removal, and Fenton tertiary treatment. The average temperature of the influent is in the range 25–30 °C, the HRT of biological sections is about 7 days and the SRT of the second biological stage is close to 30 days.

2.2. Chemical analyses

The pH was determined with a Hach-Lange's probe. The ammonium and metals were measured by means of a nitrogen analyser (TOC-L, Shimazdu) and an inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer), respectively. The Chemical Oxygen Demand (COD) and soluble Chemical Oxygen Demand (sCOD) were determined using Hach-Lange's cuvettes after filtration (at 0.45 μm). The Total Suspended Solids (TSS) were measured as the dry weight (1 h at 105 °C) of the residue of the filtered sample. The Volatile Suspended Solids (VSS) were measured as the dry weight (30 min at 570 °C) of the residue of the filtered sample.

2.3. Ecotoxicity tests

A battery of four bioassays was performed in order to evaluate the toxicity of the samples. The target organisms were: the unicellular green alga (I) *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J.Kristiansen & O.M. Skulberg (UNI EN ISO 8692:2005); the dicotyledonous plants (II) *Cucumis sativus* L. and (III) *Lepidium sativum* L. (UNICHIM N. 1651, 2003); the monocotyledonous plant (IV) *minor* L. (ISO SO/WD 20079). The samples were filtered (Whatman type 1) for the execution of algal test only. In fact, filtration was needed to avoid interference with the spectrophotometric lectures performed at the end of this test.

Each dose–response curve consisted of six dilutions (in triplicate for plant test and in quadruplicate for algal test) and the control was performed with four and six repetitions for plant and algal tests, respectively.

Significant differences between dose-effect regression lines were analysed using *t*-test ($p < 0.05$ for line slope, $p < 0.001$ for translation), for all the possible pairs.

2.4. Isolation and identification of autochthonous mycoflora

Aliquots of 1 mL of each sample were placed in Petri dishes (16 cm diam.) containing 30 mL of culture medium. Three different media were used: a modified Malt Extract Agar (MEAp) (agar 20 g, glucose 2 g, malt extract 2 g, peptone 0.2 g, water up to 1 L); Dichloran Rose Bengal Agar (DRBC 31.5 g, water up to 1 L); Wastewater-Agar (WA; agar 20