

Fungi in Waste Water Treatment

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Abstract

Traditional biological wastewater treatment produces large quantity of low value bacterial biomass. The treatment and dumping of this excess bacterial biomass, also known as waste activated sludge, accounts for about 40–65% of the wastewater treatment plant operation cost. A different form of biomass with a higher value could crucially change the economics of wastewater treatment.

Keywords: *Fungi, Waste water, Treatment, Active sludge*

INTRODUCTION

Fungi could offer this benefit in comparison with bacteria in wastewater treatment processes. The biomass produced during fungal wastewater treatment has, potentially, a much higher value than that from the bacterial activated sludge process. The fungi can be availed to derive valuable biochemical and can also be availed as a protein source. Various high-value biochemicals are generated by commercial cultivation of fungi under aseptic conditions using expensive substrates. Food-processing wastewater is

a very attractive alternative as a source of low-cost organic matter and nutrients to produce fungi with concomitant wastewater purification (Jin et al., 2001).

WASTE WATER TREATMENT

The activated-sludge process is considered, to be an efficient waste water treatment technique where the emphasis is placed on the zoological bacteria as the organisms responsible for good floe formation in an efficient treatment process. That fungi are also present has been recognized for some time, especially in

reference to one of the multiple types of bulking for which filamentous organisms are considered responsible, but it is only recently that the presence of fungi has been thought of as beneficial. Recent research was initiated in order to determine the kinds of fungi present in a small activated-sludge-type sewage-treatment plant, those techniques which would develop the fungal populations adequately, and the numbers of bacteria present simultaneously (Jin et al.,1999).

ROLE OF FUNGI IN WASTE WATER TREATMENT

A search and assessment was also made for fungi which might be allied with tertiary-treatment processes in an adjacent waste-water-renovation pilot-plant, in which the influent was the final product of the secondary plant. Fungi are indicated for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to environmental constraints. For example, *Aspergillusniger* is the prototypical fungus for the predominant production of citric, homologous proteins (esp. enzymes) and heterologous. In addition *Phanerochaete chrysosporium* is the model of white-rot fungi for the production of per oxidases . Beyond the synthesis of such relevant

metabolites, fungi have been attracting a growing and intriguing interest for the biotreatment (removal or destruction) of wastewater ingredients such as metals, inorganic nutrients and organic compounds. The major focus was therefore the use of fungi to remove or degrade various wastewater constituents (Vinalea et al.,2008). The modern biotechnological approaches like my coremediation has accelerated the use of fungi in various pace of environmental phenomenas (Sreeremya et al.,2018)

DOMESTIC SEWAGE

Domestic sewage encompasses carbon and nutrient sources that can be removed by fungal biomass. In an early investigation, demonstrated the capacities of seventeen fungal biomasses to remove phosphates (85.1%), ammonia (73.4%), total nitrogen (68.2%) and chemical oxygen demand (COD) (39.5%). They obtained fungal growth on this effluent with an accumulation of biomass (451.3 mg l-1) that contained protein (48% g g-1). There was variability in fungal capacities as to the removal of pollutants. *Trichotheciumroseum* Was the best in phosphate removal (97.6%), whilst *Epicoccumnigrum*, *Geotrichum candidum* and *Trichoderma* sp. were the best in the removal of ammonia (84.2%), total

nitrogen (86.9%) and COD (72.4%), respectively. Concerning cell-protein synthesis, *Paecilomyces carneus* had the highest ratio of protein to biomass (92.6%). Moreover, this fungus did not grow very well on domestic sewage. In invitro, domestic wastewater pretreatment by a strain of *A. niger* has been analyzed under transient conditions. This fungal biomass removed about 72% of COD and 65% of protein. Despite the differences between the bioprocess assessed in these two studies, COD and protein removal rates are in the same order. The total feasibility of domestic wastewater treatment under sewer-simulating conditions has been explored recently both experimentally and by simulation (Uduman et al., 2010). The heat treatment liquor (HTL) of an activated sludge was decolourised by *Coriolum hirsutum*. This fungal strain exhibited a strong capability to decolourise HTL (70.2%) with an accumulation of manganese independent peroxidase (MIP) and manganese peroxidase (MnP). Optimising the culture medium with the addition of nitrogen and carbon sources and improving the biomass quality resulted in increased colour removal capacity by *C. hirsutum*. Although fungal applications have shown intriguing capacities on sewage treatment, they are still underutilised in practice. This could

be delineated, in part, by a widespread apriori assumption that fungal strains do not perform as well as bacteria (Tucker, 2007).

AGRO INDUSTRIAL EFFLUENTS

Industries of olive oil, tapioca a starch, distillery (molasses), cotton bleaching, pulp and paper processing manufacture several billion litres of coloured, often lethal and harmful wastewaters over the world annually. Those effluents have strong concentrations of COD, phenol and its derivatives and often comprise of proteins, cyanides, chlorinated lignin compounds and dyes. The large amount of lignin derivatives of these effluents is majorly responsible of their dark-brown colour (Tsivileva et al., 2010). The phenolic compounds of such waste waters typically exert some bactericidal effects on wastewater treatment plant (WWTP) microorganisms. Fungal pretreatment of these effluents under aerobic conditions makes it possible to obtain phenol reduction (52-100%), good decolourisation (32-100%), biochemical oxygen demand (BOD) reduction up to 85.5%, and enzyme production (protease, Lacc (EC 1.10.3.2); LiP (EC 1.11.1.14), MnP (EC 1.11.1.13), amylase, etc.). Moreover, by optimising OMW (Olive Mill Wastewater) composition for *G. candidum* growth, from

the recent research obtained a complete degradation of phenols and 70.1% decolourisation. Since phenolic compounds causes deleterious effects to the environment, there is a great necessity to degrade phenolic compounds(Sreeremya, 2016).Some research proved that maximized colour removal from molasses wastewaters (up to 69%) with *A. niger*, after the amendment of the culture medium with co-substrate and mineral nutrients ($MgSO_4$, KH_2PO_4 and NH_4NO_3). Some of the consequences of OMW pretreatment by fungi are the 23- to 33-fold higher increases in biogas production and the fertilizing effect on plants (*Trifolium repens*) compared to non-pretreated effluent(Singh et al.,2011).

The nutritional composition of brewery wastewater provides information that it could be a suitable medium for cultivation of heterotrophic microorganisms such as fungi. Submerged cultivation of fungi is a biotechnological approach that has been explored in the past, where liquid medium is inoculated with fungal spores or mycelium from certain fungi. Simultaneous cultivation of fungal biomass and waste treatment, such as the high removal of organic carbon in brewery wastes, has also been assessed availing this

technique (Simate et al.,2011). In the last thirty years, submerged cultivation of fungal biomass has received very less attention, despite the use of filamentous fungi for wastewater treatment being identified and researched as an intriguing and interesting area with benefits such as development of a biorefinery concept and easy harvest(Shao et al.,2008).

The fungal biomass generated during treatment of brewery wastewater could also be of interest for several applications. There is a very urgent need to identify new feed resources that could aggrandize the supply of protein and thus the sustainability in animal production. Fungal biomass is rich in protein and fibres, and thus the cultivation of edible fungi in brewery wastewater in a controlled process may generate biomass that is useful as animal feed. In addition, certain fungi contain high amounts of biopolymers, such as chitin (40–45% of dry weight biomass), which make them appropriate as ingredients in biomaterial (Shannon et al.,1975). Another option is to use the cultivated fungal biomass in the treatment of polluted water, both due to the synthesis of enzymes such as lactase for certain fungal species and because it has been reported that fungal biomass can substitute

high-cost activated carbon as a biosorbent (Seluy et al.,2014).

There where research and investigation on biomass production and the removal of organic carbon, phosphorus and nitrogen from brewery wastewater through submerged fungal cultivation availing different species of fungi. From a longer-term perspective and considering large-scale in situ treatment, it is important to use non-harmful fungal species in order to avoid secondary health or environmental problems (Schnell et al.,2003).

Basidiomycota Fungi

The Basidiomycota division of fungi is a group of macro- and microorganisms uniquely characterized by the formation of basidia, a bottle-shaped cell structure containing haploid and sexual basidiospores. During their life time, most Basidiomycota fungi pass between a dikaryotic phase and diploid growth to asexual reproduction by conidia with the subsequent formation of basidiospores (Sankaran et al.,2010). Due to their environmental bond, Basidiomycota fungi have developed and optimized different systems ravished to the cycling of carbon and nitrogen sources, as well as to ecosystem balance. This caters them with a series of extracellular and intracellular

mechanisms capable of interacting with heterogeneous substrates, majorly lignin derivatives. One of the most prominent reasons for the use of Basidiomycota fungi in the degradation of PhACs is the great variety of substrates that they can metabolize (Rao et al., 2007).

The main mechanism in the degradation of PhACs and aromatic compounds by Basidiomycota is the use of the above-mentioned groups of extra- and intracellular oxidoreductases (laccases, peroxidases, CYPs, etc.) to modify or alter or break down the bonds of different compounds, mainly by extracellular pathways. Different kinds of LME and fungal mediators have been studied in relation to the biodegradation of PhACs in the white rot fungi *Phanerochaete chrysosporium*, *Phlebia chraceofulva*, *Pycnoporus sanguineus*, *Pleurotus ostreatus*, and *T. versicolor*. In all cases the participation of the CYP has a pivotal role on PhACs transformation, usually determined by indirect measurements with CYP inhibitors such as 1-aminobenzotriazole and piperonyl butoxide (Poppi et al.,2010).

Whole Cell Fungal Treatments

The recent approach of the treatment of PhACs in wastewater availing this kind of

fungi is the development of systems that could be used in real life by the design and improvement of bioreactors and the use of real wastewater from municipal, industrial or hospital effluents. Several researches, have focused on the use of whole cell Basidiomycota fungi, especially *T. versicolor*, to optimize degradation conditions as well as to apply new techniques for the monitoring of PhACs (Parenti et al., 2013). Analysis have been made of the removal rate of PhACs or endocrine disruptors using free *T. versicolor* cells in fluidized-bed, batch, and membrane bioreactors under sterile and non-sterile conditions, in which laccases and CYPs are involved. The results are satisfactory, with 72–100% effectiveness (Kapoor et al., 1995). The heterogeneous composition of real wastewater makes it difficult to implement systems capable of degrading all toxic substances or achieving 95–100% removal for all of them (Kapoor et al., 1997). Moreover, it is also necessary to assess not only the capability to remove PhACs, but also the ability to make a consistent COD and toxicity reduction of the resulting effluents, as well as the capability of the fungus to coexist with microorganisms present in the recent biological systems (Kahmark et al., 1999). In few of the research, a toxicity reduction has also been achieved; till now, no

comparisons on the efficiency on the reduction of COD in relation to conventional treatments were given. Furthermore, under non-sterilized conditions the introduced fungus was displaced by the native fungal and bacterial population (Jin et al., 2001).

Although *T. versicolor* is the basidiomycete generally availed in the biodegradation of PhACs, there is evidence of the use of other models in combination with different removal methodologies (Jin et al., 1998). For example, heterogeneous catalytic processes have been delineated with *P. ostreatus* and the use of γ -Fe₂O₃ nanoparticles in bisphenol (Jin et al., 1996). A degradation, reaching a removal rate 32–35% greater with the merging of biotransformation and Fenton-like reactions under oxidant conditions (Jimenez et al., 1997). The possibility of oozing white rot fungi and oxidative nanoparticles in the environment constitutes one of the pros of having a practical system to be applied (Jacob et al., 1998). Likewise, in the same model, the biotransformation of carbamazepine in liquid culture has been compared with solid-state fermentation on lignocellulosic substrate by evaluating the metabolic products (Jin et al., 1999). The result

analysis have proved that the metabolic pathways differed in both cases, indicating the generation of two metabolites in submerged fermentation and about 24 metabolites in solid-state fermentation, some of which being more toxic than the parent compound (Huang et al., 1996). It is important to consider that the environmental conditions have a vital influence on the degradation of the PhACs, since LME are inducible by different factors (Jin et al., 1998). Comparisons were made on the efficiency of the elimination of β -estradiol by laccases from *Pycnoporus* sp., *T. versicolor* and *Hymenochaetespreta* cultured in solid state with the addition of citric acid and lignocellulosic biomasses to optimize enzyme production (Guibal et al., 1995). The addition of these substances have profoundly improved to different levels the secretion of laccases and enabled estradiol elimination up to 80% (Gupta et al., 2000). These inductors-linked degradation could have an economic negative impact in real application as well as consequences in the operational conditions (for instance release of dark pigments) (Gomes et al., 1999).

The use of whole cell biotransformation has the merit that all the fungal mechanisms (enzymatic or not) could take

part in the process (Benny et al., 2014). For example, it was observed that the use of whole cell of *Phoma* sp., improves the release of a greater variety of compounds (including diclofenac and carbamazepine) compared with the enzymatic extract (Gomes et al., 1998). The filamentous growth could have operational problems associated (clogging, fouling and problems for biomass separation) (Beijersbergen et al., 2001). The current approach of the use of fungi is to establish the best phase for the degradation of PhACs in real wastewater and under environmental conditions, as well as to find a profitable balance of the costs of the operation systems implemented in each case (v et al., 2011).

ENZYMATIC TRANSFORMATION

The use of refined and crude extracts of enzymes such as laccases, manganese peroxidases, and versatile peroxidases have also been researched for PhACs removal, as has been reviewed by, with high rates of effectiveness of transformation (Barra Caracciolo et al., 2015).

The possibility to avoid the purification step could decrease costs and improve the adaptability; in certain cases, high amounts of crude extract should be employed to

achieve desirably high rates of conversion (Balcázar-López et al.,2016). The crude extract encompassing manganese peroxidase from *Phanerochaete chrysosporium* was assessed in the elimination of tetracycline and oxytetracycline with removal rates of 72.9 and 84.7%, respectively, in an average degradation time of about 4 h (Badia-Fabregat et al.,2014).

The use of purified enzymes allows a better control of the process and high rates of conversion of the compound in question (Badia-Fabregat et al.,2015). Different examples showed high rates of effectiveness of transformation after short reaction times with single enzymes of 10–65 min (Badia-Fabregat et al.,2017). The use of purified and combined free cross-linked enzyme aggregates composed of laccase from *Trametes versicolor*; versatile peroxidase from *Bjerkandera adusta*, and glucose oxidase from *Aspergillus niger* were featured in the elimination of PhACs (Ardao et al.,2015). The cross-linked mix proved stability under environmental and denaturing states in the elimination of acetaminophen, naproxen, mefenamic acid, indometacin and diclofenac, among others, with elimination rates greater than 82 % (Ba et al., 2014). The implementation of immobilized

laccases and peroxidases from different basidiomycetes has been tested vs. several PhACs with considerable removal rates between 62 and 100% (Aranda, 2016).

Enzymatic activities can be profoundly improved by the use of fungal redox mediators (Asha et al.,2009). Some examples of using endocrine-disrupting compounds (bisphenol A, nonyl phenol, methyl paraben, butyl paraben, and dimethyl phthalate) can be found, using different laccase enzymes from *P. ostreatus* (ATCC number MYA-2306) (Antošová et al.,2016). The influence of a synthetic (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid, ABTS) and natural (aceto syringone, AS) fungal mediators were assessed regarding the degradation efficiency of these enzymes, by boosting the degradation rate by around 50-55% (Alexopoulos et al.,1996).

CONCLUSION

Fungi of the Basidiomycota family especially *Pleurotus* sp and certain filamentous fungi helps in waste water treatment. Thus this paper concludes the fungal enzymatic activities which can be implemented in waste water treatment to avoid lethal pollutants in the waste water.

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