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Utilization of Fungi in Treatment of Sewage Water

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ABSTRACT

Conventional biological wastewater treatment generates large amounts of low value bacterial biomass. The treatment and disposal of this excess bacterial biomass, also known as waste activated sludge, accounts for about 40–60% of the wastewater treatment plant operation cost. A different form of biomass with a higher value could significantly change the economics of sewage water treatment. Fungi could offer this benefit over bacteria in sewage water treatment processes. The biomass produced during fungal sewage water treatment has, potentially, a much higher value than that from the bacterial activated sludge process. The fungi can be used to derive valuable biochemicals and can also be used as a protein source. Various high-value biochemicals are produced by commercial cultivation of fungi under aseptic conditions using expensive substrates. Food-processing sewage water is an attractive alternative as a source of low-cost organic matter and nutrients to produce fungi with concomitant sewage water purification. This review summarizes various findings in fungal sewage water treatment, particularly focusing on byproduct recovery during sewage water treatment. This review also provides an overview on performance of sewage water treatment systems under various operational conditions. Important factors such as pH, temperature, hydraulic and solids retention time, nonaxenic and axenic operation, and others that affect the sewage water treatment system are discussed. Moreover, certain important practical issues such as bacterial contamination under nonaseptic operation are also covered. The goal of the review paper is to evaluate the feasibility of cultivating fungi during sewage water treatment for deriving valuable biochemicals.

Keywords: sewage, water, fungi, treatment, purification, biochemicals, byproduct

I. INTRODUCTION

Sewage water are causing the production of large amount of toxic and stable pollutants, which are all collected into the water outcoming from the houses and industries. The disposal of these contaminated sewage into receiving waters can cause environmental damages, directly influencing the aquatic ecosystem and even human being . It stands to reason that an effective treatment of these sewage water is necessary. [1]

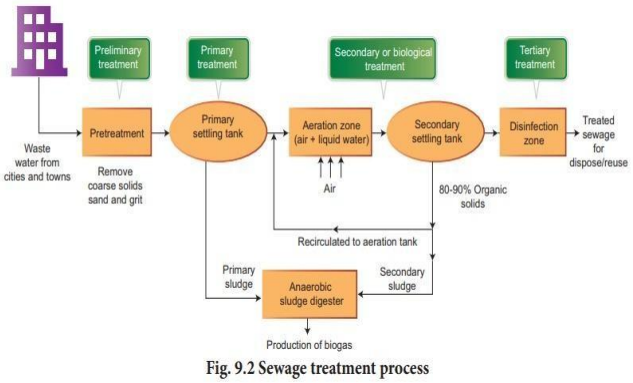


Fig. 9.2 Sewage treatment process

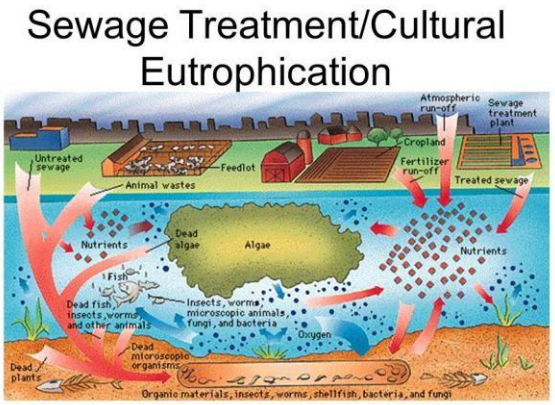
However, sewage waters are usually recalcitrant to the standard biological treatments, due to the complex aromatic compounds, the extreme

chemico-physical parameters and the presence of an autochthonous bacterial microflora . Moreover, to be competitive in the market, industries and houses should continuously update their products, strongly influencing the process itself. Consequentially, these sewage waters are very heterogeneous and complex with an inner composition which could deeply vary time by time . The harsh conditions which could be found in the sewagewater, could deeply limit even the survival of an organism. Thus, it is necessary that each new hypothesized biological treatment should combine a high efficiency with good resistance to this extreme environment. [2,3]

The final degradation yields could be improved by modifying the parameters which negatively influence the organism used. However when possible, the control of a specific parameter should be avoided. In fact, looking at the economical balance of the process, each operation means additional costs and more complex plant procedures. In the past 20 years, white-rot fungi have been applied to different biotechnological fields for their capability to degrade many aromatic compounds. However, in order to investigate this fungal potential, most of the researches have used synthetic effluents in controlled conditions. Of course, the obtained results could give little information on how a fungus could behave in a sewage treatment, competing with bacterial contamination . To date, very few experiments have faced the industrial problematic, so that nowadays the application of fungi in a plant is still a technical challenge. From an applicative point of view, a fungal free-cell treatment shows some drawbacks, since the mycelium could be too exposed to the environmental stresses. A good alternative could be the immobilization of the biomass on supports, with the aim to protect the biomass and improve the fungal activity . Confirming this, it has been observed that in some cases a supported biomass showed a higher enzymatic production compared with a free one .[4,5]

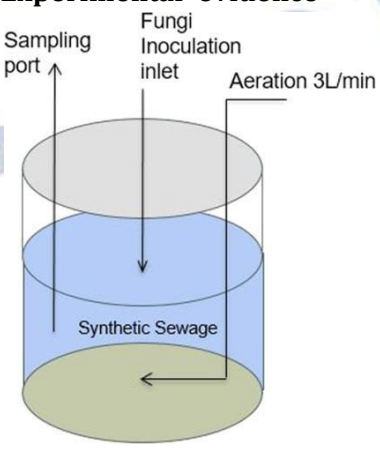
Moreover, the immobilisation of the fungus could allow the use of the system repeatedly, with obvious advantages from a further application point of view. The aim of this study was to assess if the selected strain, *Bjerkanderaadusta* MUT 2295, would confirm its potential to degrade sewage coming from houses, industries and treatment plants. Different inert supports have been tested to select the more adapt one to host the fungal biomass. The bioremediation efficiency towards sewage waters of a free-cell system and an immobilized one has been compared.

II. Discussion



The strain, *Bjerkanderaadusta* MUT 2295, is preserved .The strain was selected in this study because of its efficient wastewater treatment activity . The fungus was inoculated as twenty agar plugs (5 mm of diameter), taken from the margins of an actively growing colony on nutrient agar medium, in 500 mL flasks containing 200 mL of an high nitrogen content medium as previously described . After 7 days, the culture broths were replaced with 100 mL of T1 or P1 and the cultures were followed for 5 days. In order to compare the effectiveness of the fungal treatment with the secondary treatment used in the sewage treatment plants, flasks containing the effluent (100 mL) and 20 mL of activated sewage water, sampled in the two plants, were set up according to the standard procedures . Abiotic control (without fungal inoculum) was set up and each culture condition was assayed in 3 biological replicates. The flasks were incubated at 25 °C and 120 rpm in an orbital shaker (Infors) for 5 days[6]

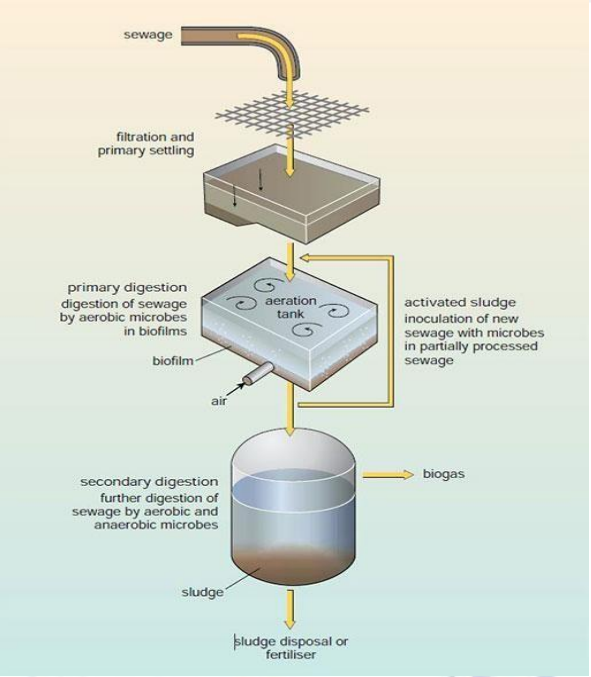
Experimental evidence



The experiment was carried out using 4 inert supports : A, circle support; B net support; C, polyurethane foam PUF (2 cm3); D, stainless steel scourers (1 cm3). Supports A and B are normally employed for activated sewage sludge immobilization and they were kindly provided by a local Engineering S.r.l., while support C was kindly provided by the Department of Civil and Environmental Engineering of the University of Delhi. The fungus was pre-grown. After 7 days, the biomass was harvested, homogenized and inoculated (5 mL) in flasks containing 200 mL of high nitrogen content medium and the different supports (60% of the volume). The carriers colonization was carried out both under agitated and static conditions, in order to define the optimal colonization condition for each support. In detail, many flasks were set up for each support and in each growth condition; after 7 days, the medium was substituted with 200 mL of low nutrient content medium, in order to evaluate the fungal stability in a poor environment. Every 2 days and for 3 cycles, 2 flasks were analyzed for the biomass resilience and the enzymatic production .[7]

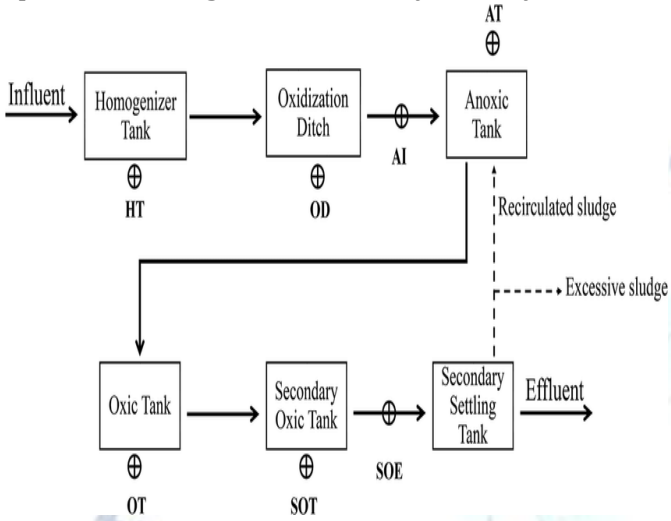
III. Results

The fungus was pre-grown as above described . The homogenized mycelium was inoculated in 500 mL flasks containing 200 mL of the same medium as free biomass (F) and in presence of twelve PUF (supported biomass, S). After 7 days, the culture nutrient media were replaced with 100 mL of more. The flasks were incubated at 25 °C in an orbital shaker at 110 rpm (F) or at 80 rpm (S) for 2 days. Since *B. adusta* is well know to produce peroxidases , during the experiments peroxidase activity was followed. Manganese-independent (MiP) and manganese-dependent (MnP) peroxidase activities were measured at 25°C, following the oxidation at 590 nm of 3- dimethylaminobenzoic acid/3-methyl-2-benzothiazolinone hydrazone hydrochloride (DMAB/MBTH), in 0.1 M succinate lactate buffer pH 4.5 . For MnP, 25 µM $MnSO_4$ was added to the reaction mixture. The enzymatic activity was expressed as international Units (U), where 1 unit is the amount of enzyme that oxidises 1 µmol of substrate per minute.



B. adusta MUT 2295 has proved to be a strong and versatile organism, able to grow in very variable and extreme conditions and effective in sewage water treatment and detoxification . [8] However, its efficiency in effluents bioremediation could be still optimized, taking into account biotic and abiotic parameters, such as nutrient addition, pH values, immobilization on different supports and agitation/static growth conditions. Their optimization and their role in the bioremediation process could give useful information for the development of industrial-scale reactor . In the first experiment, the fungus was tested towards 2 effluents, a textile and a pharmaceutical one, in order to evaluate if it was able to degrade aromatic molecules of different origins, facing diverse problems. The sewage water (T1) was highly toxicated and with a low COD. Since the organic compounds content was scarce, low amount of glucose was added in order to supply the fungal growth in the very early stage of the treatment. The pharmaceutical wastewater (P1), instead, was almost colorless and it was characterized by an elevated COD, up to 20,000 mg/L. It should be pointed out that all the experiments were carried out with real effluents and in non-sterile conditions, in order to evaluate whether the fungus was able to compete for carbon sources and nutrients with the autochthonous bacterial microflora . *B. adusta* was able to remove up to 75 % of T1 , likely by the production of oxidoreductive enzymes as peroxidases. The relation between the active fungal metabolism and the colour reduction was also confirmed by the pH data, since it decreased from 7 to 5.4. It should be considered

that many enzymes, including peroxidases, have a pH optimum among 5 and 6. It could be hypothesized that the fungus buffered the sewage water in order to maintain the external environment, as more as possible close to the optimal working ones needed by its enzymes .



Moreover, the fungal treatment was always compared with the biological one (activated sludge) already in use in the sewage water treatment of interest, in order to define if the two treatments could eventually work in a synergic way. Comparing the 2 biological approaches (fungi vs activated sludge), the results of T1 treatment pointed out the possibility to efficiently combine them together. In fact, the fungus was effective in reducing toxins but it slightly increased COD values, probably due to the release of some extracellular compounds. On the other hand, the activated sewage water was almost completely ineffective in toxin removal even though it was able to reduce up to 90 % of the COD. Thus, for a complete bioremediation process of sewage water, fungi seem to be fundamental, being active towards different sewage water components. As mentioned above, since P1 was colourless, the effect of the treatment was followed by means of the COD value, only. The fungus was able to remove up to 19,000 mg/L in 5 days (90 %).

IV. Conclusion

From an applicative point of view, immobilization could avoid several problems of dispersed cells which strongly limit the further scale-up on larger volume reactor. For example, immobilized fungal cells on supports could allow a simple reuse of the biomass, an easier liquid–solid separation and avoid clogging phenomena. [9]

Sewage effluent

Moreover, immobilized fungal cultures often showed an increased enzymatic activity compared to free biomass and they could better resist to environmental stresses due to the extreme pH values and the presence of toxic molecules at high concentration . Once the best carrier has been selected, the efficiency of the immobilized fungal biomass has been compared with the free-cell one toward sewage water treatment. An improved enzyme production is detectable when the fungus is immobilized on PUF (support C). The fungus obtained good yield of sewage water treatment (up to 60 %) and reduction of COD (48 %) and no significant differences were recorded between the 2 culture conditions.[8,9]

Sewage treatment plant

In fact, the immobilized biomass was able to completely maintain the degradation yields of the free one and even slightly improve them. Confirming what previously seen for a sewage

treatment (T1), fungal treatment was more effective , but not in COD reduction. In fact, the fungus removed more than the double of the sewage toxins, even though concerning COD , almost the opposite behaviour could be seen. Again, looking at a complete bioremediation process of sewage water treatment, the 2 biological approaches (fungus and activated sludge) had a complementary and not overlapping action, which could be fundamental for a further industrial application.

In conclusion, a very interesting fungal strain, *Bjerkanderaadusta* MUT 2295, was selected for its capability to be active in bioremediation processes, acting towards several parameters, as sewage water treatment and COD. In the future, it should be considered to evaluate the fungal potential also during longer treatment, carried out on several cycles, in order to mimic the sewage water the fungus would work in. Furthermore, the process should be scaled-up to larger volume, in order to confirm the robustness and the applicability of the system.[9]

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