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ISOLATION OF FUNGAL MICROBIAL STRAINS FROM GIURGIU NORD TECHNOLOGICAL PARK WASTEWATER TREATMENT PLANT

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Abstract

In the present study, several microbial strains were isolated in pure cultures from wastewater treatment plant of Giurgiu Nord Technological and Industrial Park, from five withdrawal points along the treatment process: point A, as the entry point of total water content after textile technological processes; point B, after colloidal particles removal stage with $Al_2(SO_4)_3$ coagulant; point C, after mechanical filtration, chemical treatment and sedimentation process stages, point D, from the water obtained after applying treatment technological stages, mixed with sewage water, plus point E, from the soil located in the vicinity of the treatment plant, characterized by high microbial load. Four semi-synthetic agarized nutritive media (PDA, MA, Czapek-Dox, Sabouroud), supplemented with 0.5% chloramphenicol for inhibition of certain bacterial species, were used for isolation of microbial load from the targeted samples. Highest microbial loads and variety were highlighted on plates isolated from soil (E samples), followed by A samples, B samples, and D samples, while C samples registered the lowest growth yield, possible due to inhibitory action of $Al_2(SO_4)_3$ coagulant. Morphological analysis of the obtained cultures revealed both filamentous fungi strains specific growth) and bacterial growth. *Isolated strains will be used in further tests, as both inactivated and viable microbial biosorbents, for removal of specific wastewater pollutants from aqueous solutions.*

Key words*: wastewater, fungi, bioremediation, textiles.*

INTRODUCTION

Water pollution control has become a great concern due to the large number of pollutants, of various proveniences, as consequence of heavy industrialization (Shannon et al., 2008).Textile industry originated wastewaters contain a complex and diverse microbiota (Maza-Marquez et al., 2016), adapted to extreme physical-chemical conditions specific to their environment. Biological materials, especially bacteria, fungi (Singh and Singh, 2014) and algae are regarded as efficient biosorbents, possessing a wide variety of functional groups like hydroxyl, carboxyl, amino, phosphate, sulfhydryl, thioether, phenol, carbonyl, imidazole moieties, and complex enzymatic equipment. Microorganisms act by sequestration and binding of a wide range of industrial wastewaters specific pollutants (Wang and Chen, 2009), decolorisation and detoxification of coloured textile wastewaters (Ma et al., 2014), which are highly toxic and pose a real thread to the environment (Anjaneya et al., 2009). Microorganisms mediated wastewater remediation has received increasing attention due to versatility and operating costs, but also stands out as environmental friendly treatment methods (Banat et al., 1996). Biosorption carried out by microorganisms has lately gained terrain as alternative efficient and cost effective treatment methods, when compared to conventional ones, like adsorption on organic resins or activated carbons (Hai et al., 2007). Fungal biomass can be used as efficient biosorbents, compared with bacterial biosorbents, due to their versatility regarding efficient use of alternative nutritive sources, with high yields of biomass (Svecova et al., 2006).

MATERIALS AND METHODS

Sample sources and isolation procedure

Water samples were collected from five withdrawal points from inside Giurgiu Nord Technological Park wastewater treatment plant: point A, as the entry point of total water content after textile technological processes; point B, after colloidal particles removal stage with $Al_2(SO_4)_3$ coagulant; point C, after mechanical filtration, chemical treatment and

sedimentation process stages, point D, from the water obtained after applying treatment technological stages, mixed with sewage water; point E, from the soil located in the vicinity of the treatment plant. Water sampling was carried out in polyethylene bottles (100mL), previously disinfected with 65% alcohol.

Nutritive media selection. Four synthetic and semi-synthetic nutritive media were selected for isolation and cultivation of microbial strains: 1) Czapek-Dox-Agar (CD) (Scharlau), semi-synthetic media with sodium nitrate as main source of nitrogen, recipe according to Thom and Raper; 2) Sabouraud-Dextrose-4% Agar (Sab) (Merck), synthetic media that allow growth inhibition of non-acidophilus microorganisms due to low pH value; 3) Malt-Extract-Agar (MA) (Scharlau), classical media for fungi growing, often used for isolation, maintenance and identification of fungal strains; 4) Potato-Dextrose-Agar (PDA) (Scharlau), selective nutritive media for fungi growing, with high content in sugars and low pH value, allow a good development of aerial mycelium. For inhibition of certain bacterial strains (both aerobic and anaerobic species), 0.5% chloramphenicol was used in the media, a thermostable antibiotic with wide spectrum.

Nutritive media plating. Both initial microbial isolations and subsequent cultivations were carried out on all four nutritive media (pH correction with 30% HCl and 5% NaOH), and poured into aseptic Petri dishes (ø90mm) in a layer of 0.5cm thickness (to ensure a corresponding humidity for the strains), and allowed to dry at room temperature (28^{o}C for 2 hours). For initial microbial isolations, 1mL of each sample was inoculated in duplicates on each nutritive media (for sample E, 10g of soil were dissolved in 100mL of sterile distilled water), and spread uniformly on the surface of the solid media. Isolation in pure cultures involved depletion of loop biomass load (carefully collected in order to avoid contamination) on the surface of the nutritive media. Incubation was performed at room temperature (30°C +/- 2^{o}C, for 10 days) for initial microbial isolations and in controlled conditions (28^{o}C, for 14 days) for the two subsequent cultivation in pure culture.

RESULTS AND DISCUSSIONS

In the isolation step microbial growth was influenced by both samples types and selected nutritive media used. On post-incubation plates carried out from inoculation of sample A (Fig. 1), it can be highlighted both development of fungal structures (including yeasts) and bacteria.

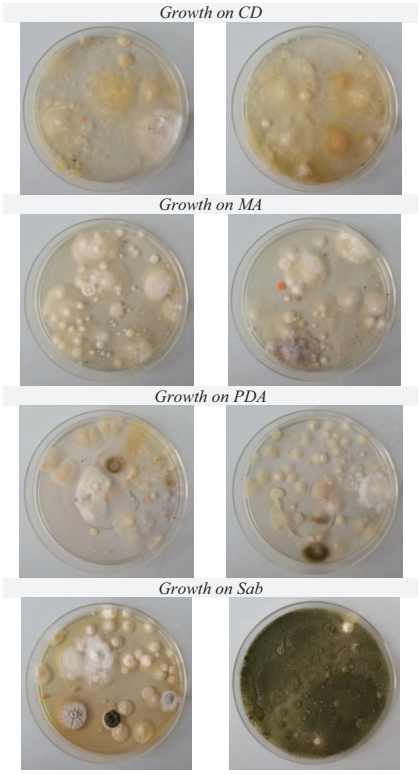


Figure 1. Sample A post incubation plates

Microbial development from sample A highlights development of both unsporulated mycelium and maturated strains, but also presence of bacterial development.

The plates inoculated from sample B wastewater (Fig. 2) show lower microbial loads when compared to sample A plates, mainly due to presence of $Al_2(SO_4)_3$ as inhibitor agent in the aqueous inoculum. CD plates show both filamentous fungi specific strains but also bacterial ones.

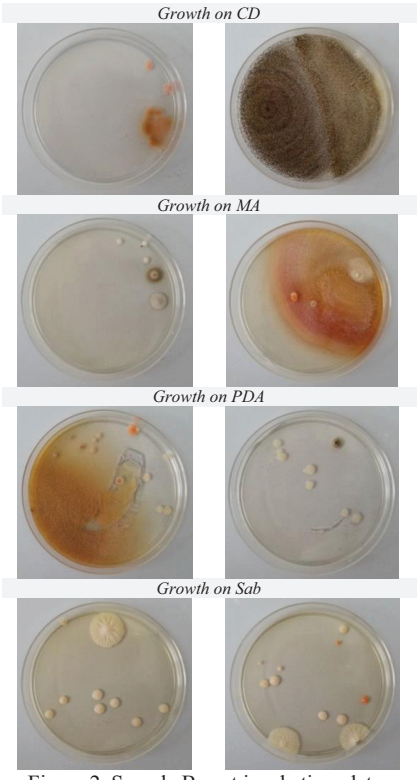
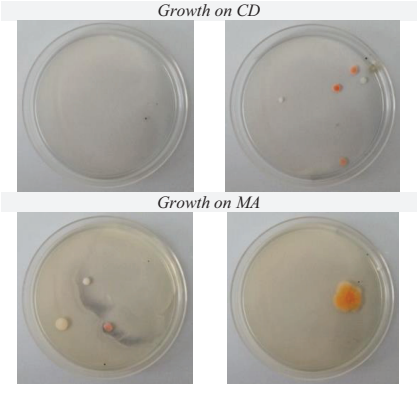


Figure 2. Sample B post incubation plates

Filamentous structures are also visible on MA and PDA plates, whilst on Sab plates both bacterial and yeast structures can be observed. Unlike plates from A and B samples, microbial load from sample C (Fig. 3) is greatly reduced, which may be caused by the action in time of the sulphate (as inhibitor) and of sedimentation process, which can mechanically bind microbial cells towards the lower part of the tank, as sampling was carried out from the upper part.



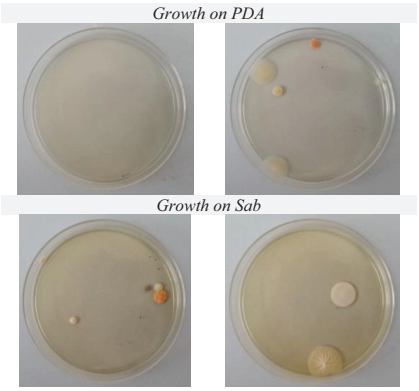


Figure 3. Sample C post incubation plates

Plates inoculated from samples D (Fig. 4), of heterogeneous composition, composed of post treatment water and sewage water, allowed the development of both bacterial and fungal species. The presence of the antibiotic in the media did not prevent the complete development of bacterial species, which may be caused either by the specificity of the antibiotic towards present strains or by high bacterial load of the samples.

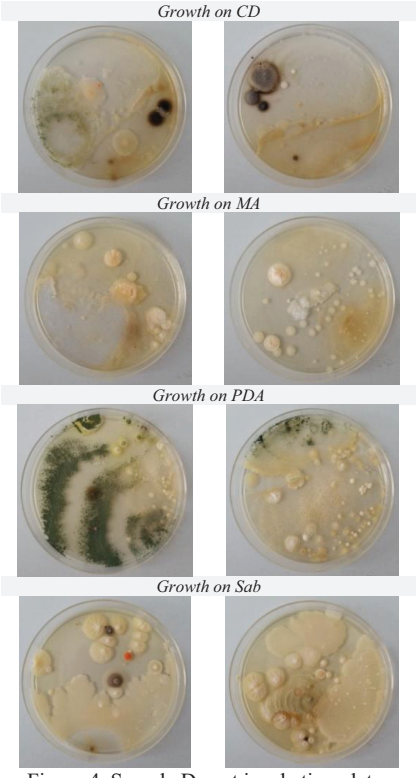


Figure 4. Sample D post incubation plates

Isolations carried out from soil sample showed strong growth of filamentous structures, these plates yielding the highest microbial load of all samples (Fig. 5). Also, it can be highlighted the development of aerial mycelium, due to significant nutritional value of used nutritive media.

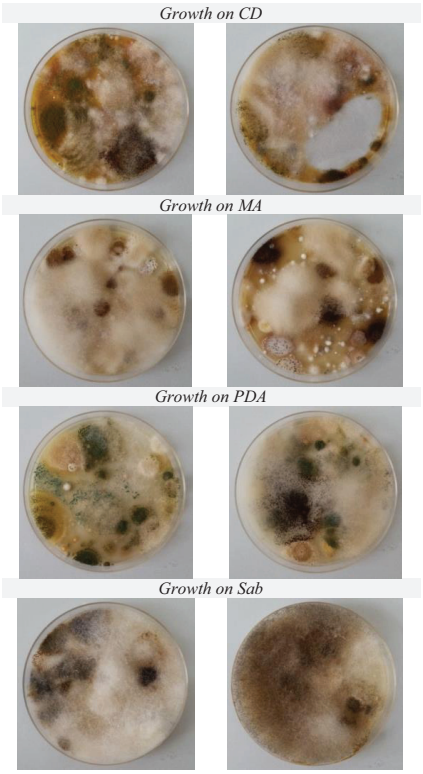


Figure 5. Sample E post incubation plates

Following isolations of main microbial species from four samples of textile industry originated wastewater and one soil sample, varying degrees of microbial growth can be observed, depending on both inoculated sample and the type of nutritive media used. The highest degree of microbial growth can be observed on soil plates, with the presence of at least four strains of filamentous fungi. From A, B and C residual water samples, the highest microbial load was present on plates from sample A and B, whilst sample C plates yielded lower microbial growth, possible due to action in time of $Al_2(SO_4)_3$, which lead to microbial inhibition, compared to sample II plates, where the sulphate was added and stirred in the water volume, with a shorter contact time. Sample D isolates also show high microbial loads. Also, beside fungal specific morphological structures, bacterial development can also be observed, despite the presence of chloramphenicol, which can be caused by both low antibiotic specificity towards respective strains and high microbial concentrations, specific to industrial wastewaters.

Furthermore, two successive isolations in pure cultures were carried out from initial isolation plates that presented significant filamentous fungi specific structures growth, which lead to an uneven number of isolates per each media, due to affinity of each strain, specific to each sample, per each media used.

From sample A isolates, only one strain was targeted for cultivation in pure cultures (Fig. 6).

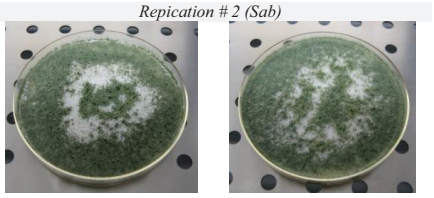
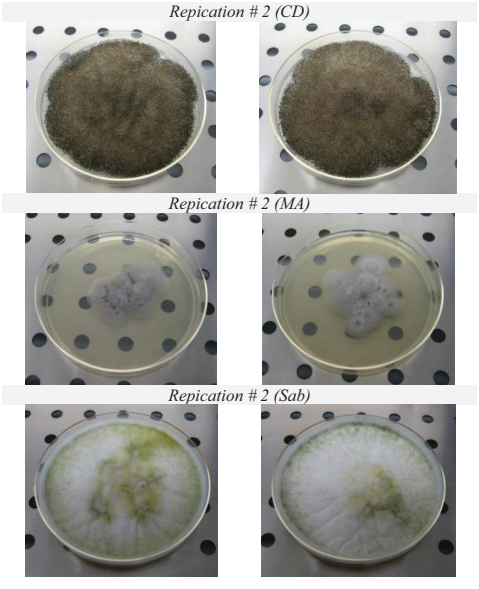


Figure 6. Sample A repication in pure cultures

Sample B repications targeted 7 strains on CD (1 strain), MA (1 strain), Sab (2 strains) and PDA (3 strains) (Fig. 7). Morphological characteristics of the obtained strains indicate filamentous fungi specific structures but also yeast specific structures (MA and PDA plates).



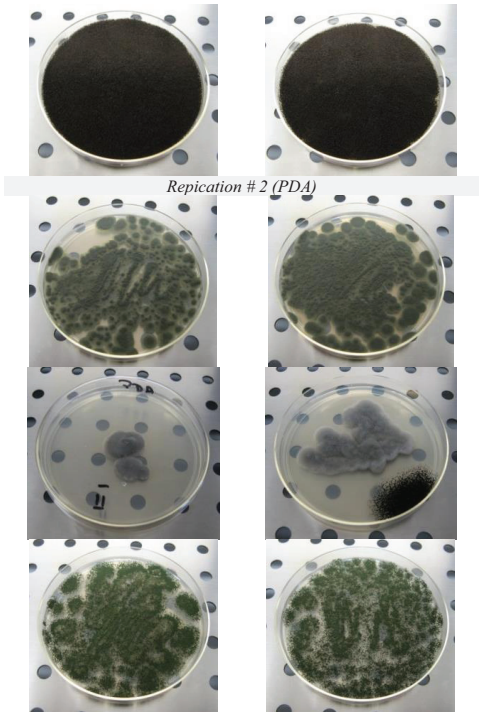


Figure 7. Sample B repication in pure cultures

Due to low bioburden of sample C plates, isolation in pure cultures targeted only one strain (grown on CD), which presents yeast like morphology (Fig. 8).

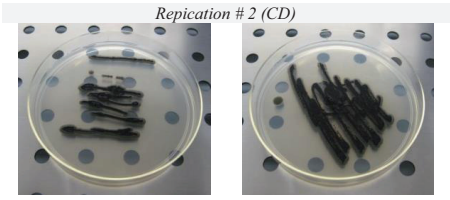


Figure 8. Sample C repication in pure cultures

Sample D platings targeted 3 strains, on CD, PDA and Sab media (Fig. 9), highlighting significant specific filamentous structures growth.

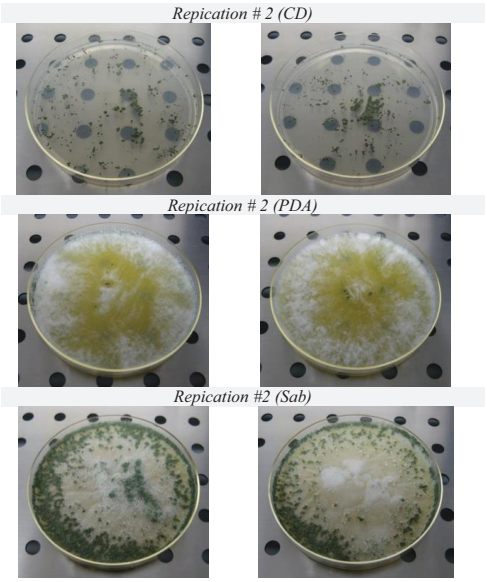
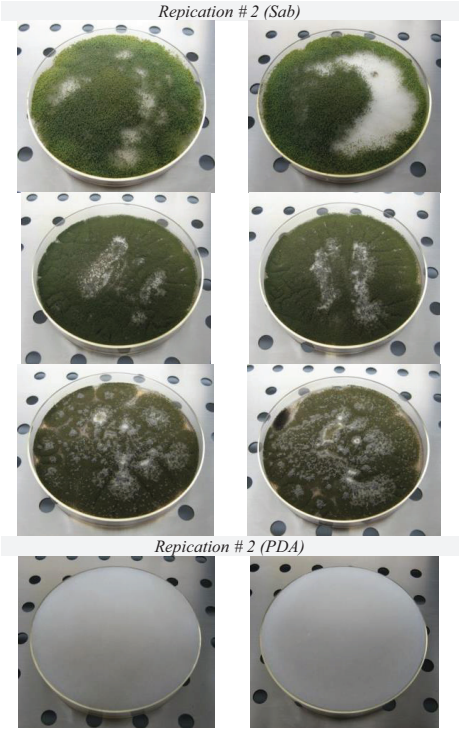


Figure 9. Sample D repication in pure cultures

Isolations in pure cultures from soil samples targeted 7 strains that yielded good aerial mycelium growth, with best growth on Sab media (3 strains) followed by PDA (2 strains), CD and MA (Fig. 10).



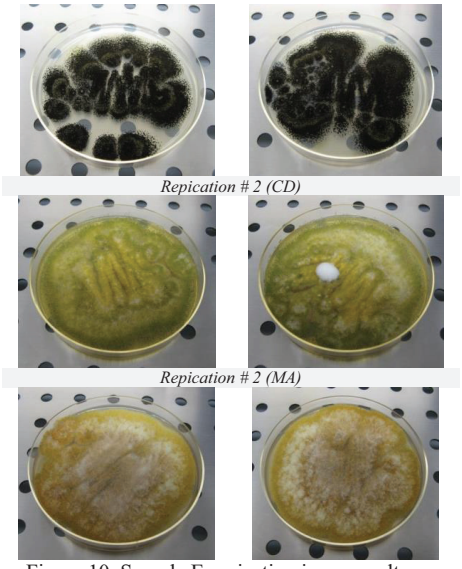


Figure 10. Sample E repication in pure cultures

CONCLUSIONS

Analysis of morphological characters of obtained microbial isolates revealed filamentous fungi and yeast specific structures, with strong development of aerial mycelium for some isolates. The strains isolated from textile processing originated wastewater will be further used for obtaining microbial biosorbents, for their active potential of bioremediation of main wastewater pollutants, backed up on their already native adaptability to presence of pollutants in the environment. Bioreactor scale biosorbents will be obtained for removal of specific wastewater pollutants from aqueous solutions: heavy metals, discoloration assays on textile dyes (Bemacid azo-dyes), BOD and COD reduction.

ACKNOWLEDGEMENTS

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REFERENCES

Anjaneya A., Santoshkumar M.,Anand S.N.; Karegoudar T.B., 2009. Biosorption of acid violet dyefrom aqueous solutions using native biomass of a new isolate of Penicillium sp. Int. Biodeterior.Biodegrad., 63, 782–787;

Banat I.M., Nigam P., Singh D., Marchant R., 1996. Microbial decolorization of textile-dye-containing effluents: a review. Bioresour Technol, 58 (3) (1996), pp. 217–227;

Hai F.I., Yamamoto K., Fukushi K., 2007. Hybrid treatment systems for dye wastewater. Environ. Sci. Technol., 37, 315–377;

Ma L., Zhuo R., Liu H., Ya D., Jiang M., Zhang X., Yang Y., 2014. Efficient decolorization and detoxification of the sulfonated azo dye Reactive Orange 16 and simulated textile wastewater containing Reactive Orange 16 by the white-rot fungus Ganoderma sp. En3 isolated from the forest of Tzu-chin Mountain in China. Biochem. Engineering Journal, Volume 82, 15 January 2014, Pages 1–9;

Maza-Marquez P., Vilchez-Vargas R., Kerckhofc F.M., Arandaa E., González-Lópeza J., Rodelasa B., 2016. Community structure, population dynamics and diversity of fungi in a full-scale membrane bioreactor (MBR) for urban wastewater treatment. Water Research, Volume 105, 15 November 2016, Pages 507–519;

Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Marinas, B.J., Mayes, A.M., 2008. Science and technology for water purification in the coming decades. Nature, 452, 301–310;

Singh A.P., Singh T., 2014. Biotechnological applications of wood-rotting fungi: a review. Biomass Bioenergy, 62 (2014), pp. 198–206;

Svecova L., Spanelova M., Kubal M. Guibal, E., 2006. Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. I. Equilibrium studies. Sep. Purif. Technol., 52, 142–153;

Wang J., Chen C., 2009. Biosorbents for heavy metals removal and their future. Biotechnology Advances, Volume 27, Issue 2, March–April 2009, Pages 195–226.