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ELIMINATION OF BIOPHARMACEUTICAL METABOLITES USING PROFUSE TECHNIQUES

Report Submitted by

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BONAFIDE CERTIFICATE

Certified that this project report titled **ELIMINATION OF BIOPHARMACEUTICAL METABOLITES USING PROFUSE TECHNIQUES** is the bonafide work of **Ms NAUFIA HASEENA N (18BBT024) and Ms SUBHASHREE M (18BBT056)** who carried out the research under my supervision for the partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology. Certified further, that to the best of my knowledge the work reported herein does not form part of any other project report or dissertation on the basis of which a degree or award was conferred on an earlier occasion on this or any other candidate.

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ABSTRACT:

Water contamination by the release of the biopharmaceuticals is one of the major environmental crises. The oceans, seas, rivers and lakes have natural mechanisms of self-cleaning that rely on plankton, which is also the base of food-chains and food-webs. They remove the waste and contaminants produced by other organisms in aquatic ecosystems, such as carbon dioxide, nitrates and phosphates. Apparently clean water may be loaded with the pharmaceutical pollutants which has a serious effect on the aquatic systems and has some serious effects the humans.It can cause various health ailments from lung to renal failure. Certain pharmaceuticals, such as ethinyl oestradiol, diclofenac and paracetamol, are known to cause endocrine disruption in the organisms. Traditional wastewater treatment technology has a significant impact but does not guarantee the removal of effluents of high quality, so some advanced studies and techniques show greater much desirable effect by removing the metabolites from water. In the first study, the performance and efficiency of free and immobilised cells of a microalgae *Nannochloropsis* sp. in removing the pharmaceuticals was studied. This species is chosen for their occurrence or persistence in the environment that is in vicinity to the waste that is discarded. The drugs chosen are paracetamol, ibuprofen, olanzapine and simvastatin. Both cells, free and immobilised, respond differently to each pharmaceutical. In the second study, laccase, an oxidative copper (II)-enzyme, was used as the basis for the degradation of phenol-targeting pharmaceutically active compounds was researched and studied. In the third study, the electrocoagulation method to remove the heavy metals pollution from the contaminated water bodies

was studied. Various techniques such as physical, chemical, biological, advanced oxidation and electrochemical processes were used for the treatment of domestic, industrial and agricultural effluents.

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INTRODUCTION:

Water pollution is one of the most terrifying environmental crises, prevailing around the world. Since clean water is a vital resource for every living organism on the planet, it is the need of the hour to conserve it. Pharmaceuticals and their metabolites are let free into the aquatic environment by domestic, hospital, and pharmaceutical industry wastewaters. Anthropogenic contaminants reach water bodies through different pathways: direct sewage effluent discharges, via rivers, urban and agricultural run-offs, dumpings, atmospheric transport, and various other means. Pharmaceuticals and their metabolites are also excreted through urine and faeces into sewage. The metabolic path of some pharmaceuticals, containing up to 95% of the active ingredient, may involve excretion in the original form. These are eventually discharged into water bodies through municipal wastewater. These contaminants in the water may cause various diseases in humans and the aquatic ecosystem. Bioremediation using microalgae presents several advantages, including high biomass productivity, high carbon uptake and bioconversion, not requiring large areas of land, and a biorefinery concept that can be used to produce chemicals from the microalgal biomass.

PROBLEMS IDENTIFIED:

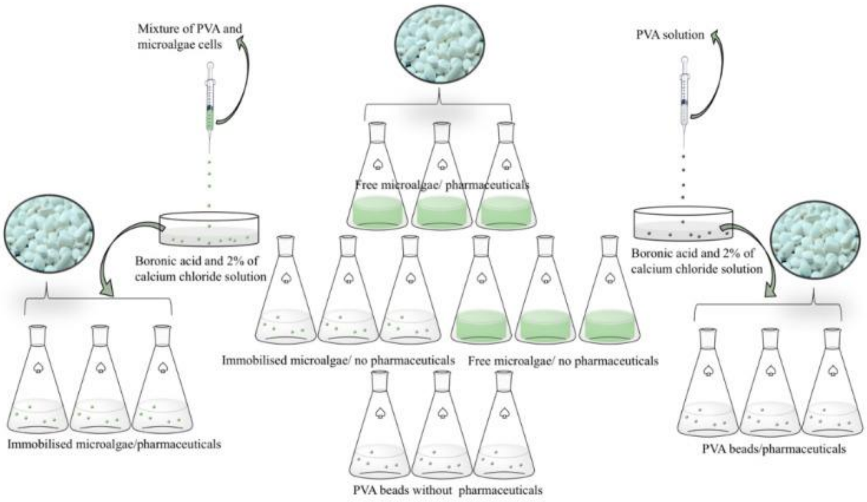
Given the low concentration of drugs in wastewaters and their nature, such as hydrophilicity, solubility, volatility and biodegradability, the conventional wastewater treatment plants are not, in general, effective for their removal. So, immobilised algae and other techniques may pose as a potential solution to overcome this problem. It is well known that microalgae remove nitrates and phosphates from wastewaters. The majority of the bioremediation studies focus on the removal of nitrates, phosphates and heavy metals from wastewaters and sewage effluents. The detected amount of these antibiotics in waste water is very minute but they are highly toxic for human, animal and aquatic lives even at very low concentration and cause health problems like headache, diarrhoea, tremors, nausea, vomiting. It can cause various health ailments from lung to renal failure. Certain pharmaceuticals, such as ethinyl oestradiol, diclofenac and paracetamol, are known to cause endocrine disruption in the organisms.

POSSIBLE SOLUTIONS:

1. **ELIMINATION OF MEDICAL WASTES USING IMMOBILSED ALGAE:**

Immobilization Procedures:

The immobilization of the microalgae was achieved through the formation of beads of PVA gel. A 24% PVA solution was first prepared and dissolved with continuous stirring at about 50 °C for two hours. Then the centrifuged microalgae were added to the PVA solution in a 1:2 portion. This mix was dripped into a solution of boronic acid and 2% of calcium chloride in which the beads formed. IN the preparation of the beads, 2 mL of PVA and 1 mL of microalgae cells yielded approximately 25 beads, each ca. 4 mm in diameter. The chromatographic analysis of PAR, IBU, OL, SV and SVA was carried out. The method for PAR, IBU, OLA, SIM, and SVA quantification was validated according to the standard procedures and guidelines with respect to system suitability, linearity, accuracy, precision, recovery, limits of detection and quantification, selectivity and specificity. The results showed that free microalgae cells remain alive for a longer time than the immobilised ones, suggesting the inhibition of cell proliferation by the polymeric matrix polyvinyl alcohol.



2. IMIDAZOLE-RICH COPPER PEPTIDES AS CATALYSTS IN XENOBIOTIC DEGRADATION:

Laccase, an oxidative copper (II)-enzyme, was used as the basis for the degradation of phenol-targeting pharmaceutically active compounds in this research. Copper (II)-peptides designed to mimic the structure were synthesized and characterized. The apo-peptides and their Cu-peptides enhance the oxidation activity of laccase and provided an environmentally friendly alternative using fewer chemicals and producing less harmful by-products. Being the smallest organometallic catalyst among the others reported herein, TpCuS 12 is the best catalyst and shows promising potential for use in environmental applications for the removal of the biopharmaceutical wastes.

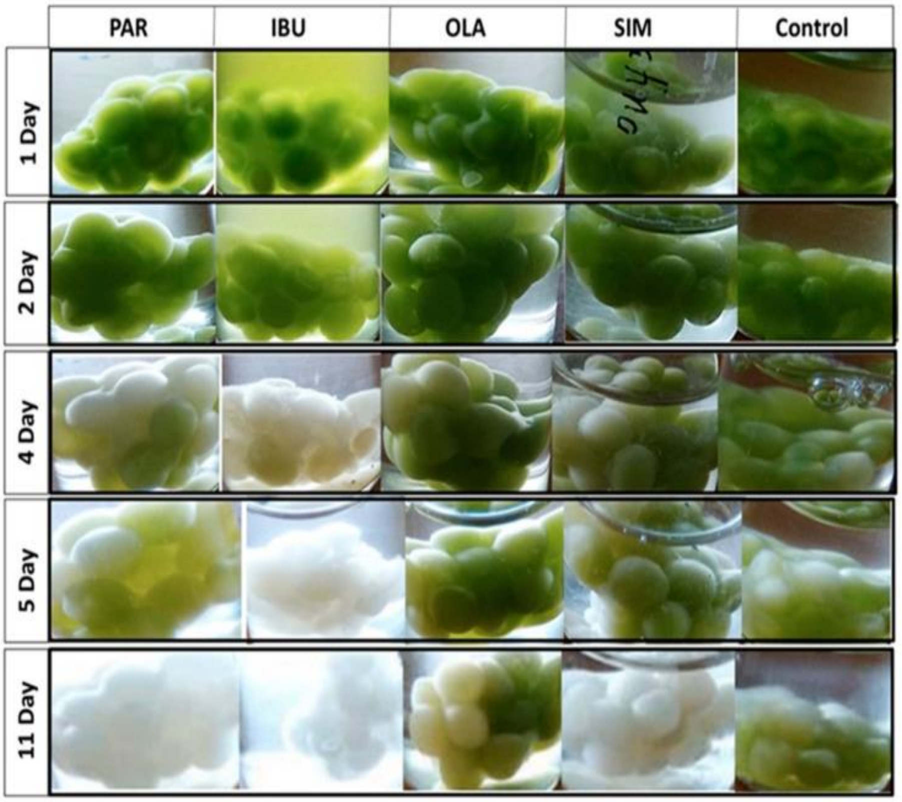
3. HEAVY METALS REMOVAL FROM AQUEOUS ENVIRONMENTS BY ELECTROCOAGULATION PROCESS:

Electrocoagulation is an attractive method for the treatment of various kinds of wastewater, by virtue of various benefits including environmental capability, versatility, energy efficiency, safety, selectivity and cost effectiveness. The process is characterized by simple equipment, easy operation, less operating time and decreased amount of sludge which sediments rapidly and retain less water. The removal efficiency also depends upon the angle of the electrodes. Efforts should be made to study the phenomena of electrode passivation to reduce the operating cost of the EC process. Many efforts were made to perform EC experiments at pilot plant scale level using real biopharmaceutical waste to explore the possibility of using EC for treatment of real medical waste.

CONCLUSION:

From the first study, the results demonstrate the effectiveness of *Nannochloropsis* sp. free cells for removing olanzapine and *Nannochloropsis* sp. immobilised cells for removing paracetamol and ibuprofen. There are already companies that are successfully using microalgae technology to remove heavy metals or to recover nutrients from wastewater, adding value to the resulting biomass. High capital investment and operating costs are the most important disadvantages associated with bioremediation technology.

In addition, *Nannochloropsis* sp. is a promising biobased feedstock, rich in lipids, carbohydrates and proteins and it is renewable.



From the second study, though laccase reported some activity in the degradation of insulin, diclofenac, celebrex, orphenadol, heparinol and cetirizine, the degraded products formed from these PhACs are cresols which were degraded using Tp-CuS 12. TpCuS 12 is one of the best catalysts and shows promising potential for use in environmental applications. From the third study, electrocoagulation is one of the best treatment processes that is capable of being an effective treatment process for the removal of the toxic chemicals from the biopharmaceutical wastes that are let into the water bodies.

RECOMMENDATIONS:

From the results obtained, it became apparent that the microalgae *Nannochloropsis* sp. could be a promising species in the removal of pharmaceuticals from effluents. *Nannochloropsis* sp. possesses a high growth rate, resilience, adaptation to a wide range of growth media, halotolerance, and accumulation of large amounts of lipids, which is an advantage as it adds value to the biomass produced during the bioremediation process.The removal of paracetamol and ibuprofen by *Nannochloropsis* sp., after 24 h of culture, was significantly higher in immobilized cells. It has been found that free cells show a better performance on the removal of olanzapine, suggesting more affinity with this molecule than to paracetamol and ibuprofen. Fluorescence microscope observations showed the internalization of olanzapine in living cell. Free cells removed a significantly higher concentration of olanzapine than immobilised ones, suggesting a higher affinity to this molecule than to paracetamol and ibuprofen.

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