Review Article

Characterization and Optimization of Bacterial Biofilm in Secondary Wastewater Treatment

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ABSTRACT

Research at the Division of Environmental Microbiology has helped identify possible bacterial key-organisms with efficient nutrient removal traits during the past few years. The retention, growth, and maintenance of biological activity within the procedure are necessary for the efficient utilisation of these organisms for improved nutrient removal in secondary wastewater treatment applications. This may be done by immobilising the organisms using the right technique. (e.g- Trickling filter). Locate the microorganisms that are generating biofilm during secondary wastewater treatment and separate them from those sources. As part of earlier research efforts at the Division of Environmental Microbiology, a variety of effective nutrient-removing bacteria have been identified from various wastewater treatment settings. The initial step is always to grow the chosen microorganisms on a surface substrate. This is done with the intention of studying the time-course of biofilm development, interspecies interactions, the matrix composition, or the genetic expression of biofilm bacteria. Following water treatment, the amount of BOD and COD will be evaluated. The mechanisms of initial adhesion, biofilm development throughout time, dynamics and properties of extracellular polymeric substances (EPS) and exopolysaccharides, nutrient removal activity1, and the influence of bacterial interactions were studied. The results demonstrated that if a proper nutrition supply was provided, all of the tested bacterial strains could form a single strain biofilm and then check the level of BOD and COD. This study emphasised the stages in producing single or mixed strain biofilms as well as the importance of interactions on biofilm functionality. The information presented here can be used to model biofilm systems and as a tool for choosing bacterial strains and use it in a wastewater treatment process.

Keywords: Biofilm, Secondary wastewater treatment, BOD, COD, Exopolysaccharides.

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INTRODUCTION

Despite the fact that all known living forms require water, ecological destruction and water pollution are both serious issues. Water pollution has become a major worldwide concern as a result of industrialization, globalization, population expansion, urbanization, and conflict, as well as increased luxury and lavish lifestyles. Eutrophication of lakes and the ocean, brought on by nutrient runoff from industries, agriculture, and human activity, poses a hazard to the preservation of biodiversity and public health. Therefore, biological wastewater treatment is crucial for the health of our water bodies.[3]

Many countries were devastated by cholera and typhoid fever epidemics that broke out in the middle of the 19th century. Sewer systems were built in a number of major cities as a result of the growing understanding of the function that microorganisms and sanitary systems play in the transmission of disease.[1] A need for wastewater treatment arose in the late 19th century as a result of the serious contamination of rivers and lakes caused by the enormous population growth in urban areas. The primary treatment—screens, grits, strainers, and settling tanks—was the principal component of the first treatment facilities utilised in Europe.[2]

The primary treatment—screens, grits, strainers, and settling tanks—was the principal component of the first treatment facilities utilised in Europe. As early as the 1880s, a full-scale biological treatment plant using the biofilm technology (trickling filter) was functioning in the United Kingdom, which was at the time the world leader in water treatment.[1] Throughout the first half of the twentieth century, activated sludge technology and modified forms of the trickling filter were incorporated into Europe's secondary biological wastewater treatment systems.[2]Diverse strategies and procedures that disrupt bacterial adhesion, bacterial communication systems (quorum sensing, QS), and **Biofilm** matrices have been used to eradicate hazardous biofilms. However, biofilms also have useful applications in a number of industries, such as plant protection, bioremediation, wastewater treatment, and corrosion inhibition, among others.[5]

BIOFILM

A biofilm is an assembly of microbial cells with a surface attachment which is contained in a matrix of extracellular polymeric molecules.[4]

Typically, microorganisms were supposedly discovered in 1684 by Antoni van Leeuwenhoek, the first person to publish microscopic observations of microorganisms. He can be attributed with the discovery of microbial biofilm after making the initial observation of bacteria on tooth surfaces using a simple microscope. The earliest recorded observations of microbes "connected in layers" were not made until the 1940s, despite the fact that surface-associated communities are where microorganisms thrive most frequently on Earth. Research on "microbial slimes" accelerated in the 1960s and 1970s, although the name "biofilm" was not widely adopted until 1984.[3] Over time, different interpretations of the term "biofilm" have been put forth. According to the all-knowing encyclopaedia Wikipedia as a biopic is "a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface".[3] Biofilms can appear in the form of dental plaque, slippery stream rocks and pebbles, slimy coatings in showers or on boat hulls, pus on infected wounds, or the mass obstructing water distribution pipes in your daily life. Extracellular polymeric substances (EPS), which are created by bacteria in biofilms and serves as the scaffolding for the structural matrix of the biofilm, are what keep cell aggregates together.[2] Even though it consumes a lot of energy, the production of EPS under growth-restrictive conditions highlights the benefits of being in a biofilm for bacterial cells. The biofilm matrix shields the bacterial cells from stressors like antimicrobials and the environment by acting as a physical barrier.[2]

Need of Biofilm to Treat Wastewater

Compared to suspended growth systems, biofilm systems for wastewater treatment provide a number of advantages. The advantages of biofilm treatment processes include operational flexibility, a small footprint requirement, faster hydraulic retention times, resistance to environmental changes, longer biomass residence times, higher active biomass concentrations, improved ability to break down recalcitrant compounds, and a slower microbial growth rate, which results in less sludge production.[11]

In the process of treating wastewater, biofilms use a variety of removal processes, including biological degradation, biosorption, bioaccumulation, and biomineralization. Components of the biofilm matrix have been discovered to efficiently biosorb both heavy metals and organic solvents.[10] Reactors that utilise the natural microbial flora or specific strains of microorganisms may get rid of items like mixed pharmaceutical industry effluent, n-alkenes, carbon tetrachloride, and chlorophenols.[11]

Constituents of Bacterial Biofilm

Bacterial biofilms are groups of bacteria that have formed a self-made matrix and are affixed to a surface or to one another. The biofilm matrix is made up of polysaccharides, proteins, and fibrin (like alginate), and eDNA. Bacterial biofilms are complex bacterial colonies that attach to surfaces and are kept together by an extracellular polymer matrix that is mostly composed of polysaccharides, proteins, and DNA.[7]

The formation of bacterial biofilm is a complicated process and can be delineated in five main phases:

(i) Revocable attachment phase, during which bacteria attach to surfaces in an unspecific manner.[9]

(ii) During the irreversible attachment phase, lipopolysaccharide and fimbriae, two bacterial adhesins, engage between bacterial cells and a surface (LPS).[8]

(iii) Production of extracellular polymeric substances (EPS) by local bacterial cells.[9]

(iv) Biofilm maturation stage in which bacterial cells produce and release signalling molecules in order to recognise one another, resulting in the creation of microcolonies and biofilms.[9]

(v) The dispersal/detachment phase is when the microbial cells return to their independent being lifestyle after leaving biofilms. The development of biofilms is detrimental to the food, beverage, medical, marine, and other sectors.[8]

Mechanism of Production

Biofilm formation and development is a fascinatingly complicated process that involves altered genetic genotype expression, physiology, and signal molecule-induced communication. In the majority of moist environments, biofilms can form on biotic or abiotic surfaces. There are several unique phases that are vital to the biofilm generation process, and the most important ones have been simplified.[6] Aquatic surfaces often develop a conditioning coating of adsorbed organic and inorganic compounds.[7] Chemotaxis or Brownian motion are two methods by which bacteria move toward the surface. As a result, the bacteria briefly interact with the surface. Van der Waals forces, for example, are general interacting forces, electrostatic forces, hydrogen bonds, and Brownian motion forces are responsible for this. The cells will be firmly attached to the surface by the extracellular polymeric molecules that develop there. Although this situation is occasionally referred to as having an irreversible attachment, only when there is no physical or chemical stress is this true. Two common techniques for attaining long-lasting attachment are the release of specific macromolecule adhesins that aid molecular binding or exopolysaccharides that form complexes with the surface material.[10] A significant component of these proteinaceous adhesins are the sheet-rich, water-insoluble amyloid fibrils seen in 5-40% of the strains in both freshwater and wastewater treatment biofilms. The initial attachment is influenced by a variety of short-range forces, including hydrophobic contacts, covalent, hydrogen, and ionic connections, among others.[14]

Due to repelling electrostatic forces, the initially attached cells seldom ever make direct contact with the surface; instead, secreted polymers bind the cells to the surface substratum. Relatively quickly, connection goes from being reversible to being irreversible.[12] Numerous studies claim that solid attachment occurs in a matter of minutes or less. After becoming fixed to the surface, the biofilm community grows and develops, or matures, as a result of the recruitment of planktonic bacteria and cell division. [16] Microcolonies are created when surface-attached bacterial cells multiply and make additional EPS using nutrient content of the conditioning film and aqueous bulk.[17] The microcolonies eventually grow to create a layer that covers the surface. When compared to planktonic cells, the gene expression pattern differentiates during biofilm formation.[17] The formation of surface appendages involved in bacterial movement is down-regulated due to the immobility of cells in the biofilm matrix, but the generation of EPS and membrane transport proteins such porins is up-regulated.[15] Quorum sensing, a signal molecule-driven communication mechanism, controls population density and the up- and down-regulation of genes.[16]

Mature microorganism biofilms are dynamic, geographically and temporally diverse communities that can adopt various topologies depending on the surrounding environment (nutrient convenience, pH, temperature, shear pressures, osmolarity) and the makeup of the microbic consortium. Complex structures, such as mushroom-like towers surrounded by exceptionally pervious water pathways, aid in the passage of nutrients and atomic number 8 to the interior of biofilms, square measure commonly identified.[17] The biofilm development method is fairly slow, many days square measure usually needed to succeed in structural maturity.[18] A mature biofilm is a lively structure with a complex organisation that constantly adjusts to the environment, which implies that under bad conditions, bacteria may leave their sheltered life within the biofilm community in search of a different, more hospitable habitat to cool down in. This is referred to as detachment.[19]

Significance of Secondary Wastewater Treatment

The process of removing perishable organic materials (in solution or suspension) from waste or other supplies of waste product is known as secondary waste product treatment.[20] The goal is to reach a specific level of effluent quality in a highly efficient waste treatment facility that is suitable for the intended disposal or utilisation potential.[21] Secondary treatment is frequently preceded by a "primary treatment" process in which settleable materials are removed via physical section separation. Biological processes frequently remove dissolved and suspended organic materials during secondary treatment, as determined by organic chemistry atomic number 8 requirement (BOD).[22] Microorganisms carry out these processes in either an extremely controlled aerobic or anaerobic process depending on the treatment methodology. [23] Perishable soluble organic pollutants (such bacteria and protozoa) are eaten by theseorganisms.[99]

In secondary wastewater treatment methods, microorganisms are utilised to biologically remove contaminants from wastewater. Both aerobic and anaerobic secondary biological activities need different bacterial populations. Under specific conditions, coupled anaerobic-aerobic processes may also be used.[24] There are so many processes are used in the secondary wastewater treatment like: aerobic, aerobic lagoons, activated sludge, rotating biological contactor, anaerobic.[25] But in this project method we used the trickling filter.[26] The description and the working process of the trickling process are underneath[100]: -

Trickling filter: A mechanism wherein wastewater is dispersed over a fixed bed of material such as rocks, gravel, plastic substrate, and so on. The wastewater runs downhill over the media surface, where microorganisms develop a layer of biomass and consume water pollutants.[27]

Design throat: This kind of filter is approx 1 to 2.6 m deep, however filters filled with lighter plastic infill may reach depths of up to 12 m.[28]

The perfect filter material has a high surface-to-volume ratio, is light weight, affordable, and long lasting. When it is available, crushed rock or gravel is the least expensive option. The particles should have a diameter of 6 to 10 cm, with 90 to 95% of them being homogeneous.[29] Typically, a material having a specific surface area of 91 to 150 m2/m3 for plastic packaging and 46 to 60 m2/m3 for rocks is used.[30] Larger pores (such those found in plastic packaging) provide for improved airflow and are less likely to clog. In order to prevent congestion, primary treatment is also necessary.[31]

Adequate air flow is critical for ensuring adequate treatment performance and preventing odours. The underdrains ought to provide an air conduit even when the loading rate is at its highest.[32] Effluent and additional sludge can be collected using a perforated slab that anchors the filter's bottom.[33] To encourage wetting and flushing of the filter material, the effluent loop pattern is frequently used in the construction of trickling filters.[34]

With time, the connected layer loses its ability to remain attached and sloughs off when the biomass thickens and the attached layer becomes oxygen-deprived. Under situations of maximal loading, sluffing will also occur.[36] The collected effluent has to be cleaned in a settling tank to get rid of any biomass that could have gotten loose from the filter. [37] The wastewater characteristics, filter media type, ambient temperature, and discharge requirements all influence how much wastewater may be put to the filter (i.e., the hydraulic and nutritional loading rate).[38]

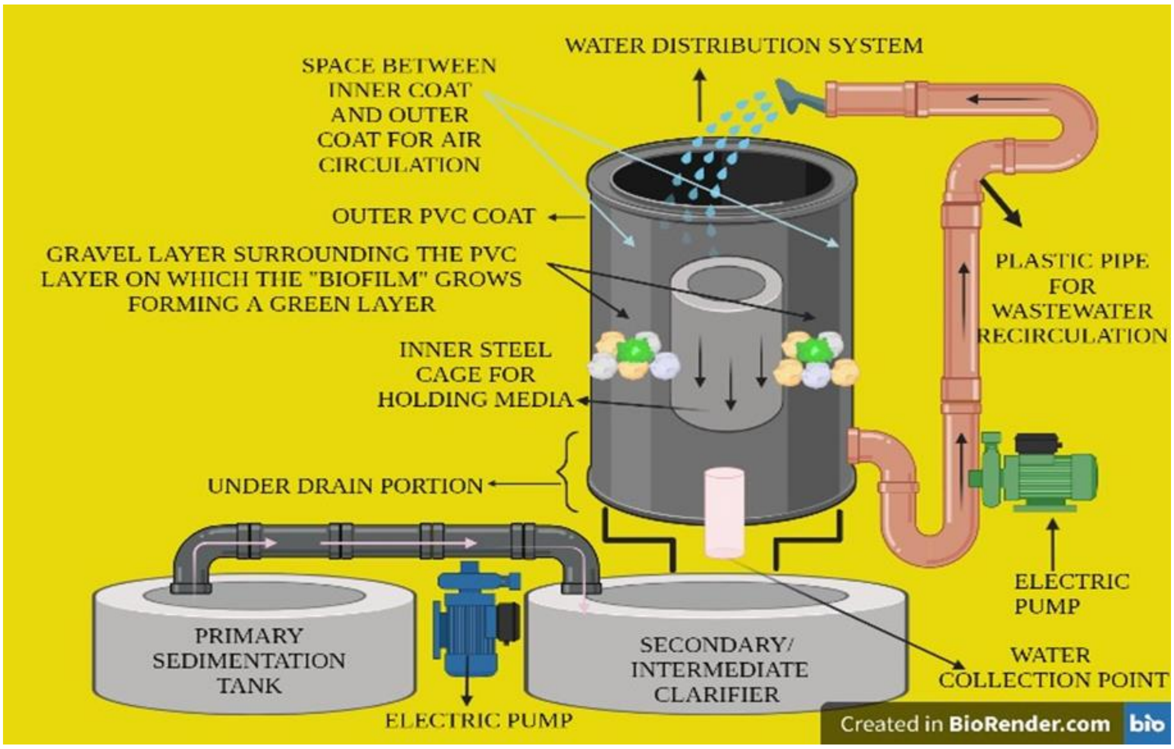

Figure 1: Design and the working process of Trickling Filter

Overview of the working method: A biological reactor with a fixed bed that operates mostly under aerobic conditions is referred to as a trickling filter.[39] Pre-settled wastewater is continuously sprayed or trickled over the filter. The bio layer that covers the filter material causes aerobic destruction of organics when water passes through the pores. Pollutants are removed from wastewater using trickling filters by filtration, adsorption, and assimilation.[40] Wastewater should flow over the medium in a thin layer to give time for treatment. The medium serves as a substrate for the development of a biological film that is fed by the wastewater's nutrients.[41]

Execution and Maintenance:

1. In the event of a failure, a skilled operator must monitor the filter and repair the pump.[42]
2. The sludge that builds up on the filter has to be regularly removed in order to avoid clogging and maintain a thin, aerobic biofilm.[43]
3. To flush the filter, high hydraulic loading rates (flushing dosages) might be utilised. The field operation should be used to determine the best dose rates and flushing frequency.[44]
4. Moisture must be maintained in the packaging. This might be an issue at night when the water supply is decreased if there are power outages.[45]
5. Common problems with trickling filters include filter flies and snails eating the biofilm, which must be dealt with by backwashing and periodic flooding.[46]

Boons of biological Trickling Filter: There has so many benefits of using the trickling filter, those are:

i. **Design compatibility:** Biological trickling systems have a very simple design, consisting of just a few of pieces, such as a device or tower filled of filtering media such as stone, gravel, or sliced plastic, as well as a distributor (usually a rotating arm with sprinklers), as well as an under-drain system.[93] Because biological trickling systems square measure quite easily from a mechanical standpoint, they will be somewhat easier and more cost-effective to repair and maintain than alternative effluent treatment technologies.[47]

ii. **Small footprint**: A trickling filter system employs particle filtering material with a high surface area. As a result, the medium can hold a greater amount in a smaller space. As a result, biological trickling systems may be an excellent fit for urban areas or other locations where larger tracts of land are either too expensive or simply unavailable for the construction of typical wastewater treatment ponds.[48]

iii. **Good organic deportation:** Reduce the organic content of a wastewater stream, which is usually quantified as biological oxygen demand, using biological trickling filters (BOD). In streams with moderate to high amounts of organic material, biological trickling filters are good for quickly reducing BOD levels. They can also remove some suspended particles.[49]

iv. **Energy intensity:** The effectiveness of a high-rate trickling filter is determined by the organic loading rate as well as the recirculation ratio. There are numerous equations available for estimating plant efficiency based on organic loading rate and recirculation ratio.[44] Most aerobic systems will require considerable energy consumption for aeration, agitation, or system pressure maintenance, whereas trickling bio filters will only require energy to pump water to the distributor mechanism.[32] As a result, biological trickling systems often use less energy than other aerobic treatment technologies. If your municipal facility wants to decrease its energy use, biological trickling systems could be a great solution.[50]

Drawbacks of the Biological Trickling Filter

I. **Problem of clogging:** When solids enter the system and become entangled in the distributor mechanism, filter medium, or underdrain system.[51] Clogs can impair the normal flow of air and water through the system., reducing system capacity and threatening the biofilm by depleting it of the circumstances required for optimum development and function.[52]

II. **Unbiddable flow rate:** Due to the clogging problem flow rate can be inflexible. Otherwise, A sustained flow of water through the system is necessitated via biological trickling filters.[54] This keeps the biofilm wet and aerated all the times, enabling it to Continue to perform as planned. The system is subject to filter fly infections if there is insufficient moisture. [55] Sections of the biomass may become anaerobic when saturated with additional organics products, resulting in unpleasant odours and excessive sloughing of biofilm.[56]

Applications:

- Biofilm, as was already noted, is crucial to the treatment of wastewater. Additionally, it has several uses in a range of industries, including plant protection, bioremediation, and corrosion inhibition.[57]
- Beneficial application of biofilm in the food and agriculture industries.
- Application of biofilm in microbe-assisted bioremediation.[58]
- Biofilm Reactors for Microbial Fermentation-Based Value-Added Product Production.[57]
- The Use of Biofilm-Forming Bacteria to Improve Organophosphorus Fungicide Degradation.[57]

Microbial strains which are used to remove pathogen and parasites in trickling filter [89]

Strains	Removal (%)
Salmonella	75-94
Enterovirus	56-95
Bacterial phage	40-91
Entameoba histolytica	74-90
Fusarium oxysporum	92.5

- After a specific time of development, *Paramicrosporidium saccamoebae* took the position of the fungus Fusarium.[60]
- The broad distribution of the functional genes for nitrogen removal ("amoA, nirK, nirS, napA, narG, and nosZ") in MTF suggests that the simultaneous nitrification and denitrification (SND) mechanism is a crucial factor in nitrogen removal.[61]

Microorganisms used in the biological treatment of wastewater:

Species	Genre	Process involved
Achromo bacter	Bacteria	Biofilters and activated sludge [80]
Alcaligens	Bacteria	Biofilters, activated sludge, and sludge [81]
Bloodworm	Metazoa	Biofilters and treated sludge [80]
Flavo bacterium	Bacteria	Activated sludge, biofilters, sludge digester [80]
Geotrichum	Geotrichum	Activated sludge and biofilters[80]
Micrococcus	Bacteria	Activated sludge and biofilters [80]
Tubifex	Metazoa	Biofilters [80]
Reference: https://www.climate-policy-watcher.org/wastewater-treatment/important-microorganisms-in-wastewater-bacteria-and-fungi.html		

Recent advancement of research on biofilm production using microbial consortium:

In recent years, attached growth methods for wastewater treatment have considerably improved. For the goal of removing organic matter (BOD) and pathogenic contamination, their applicability can be expanded to sustainable municipal wastewater treatment in rural areas and underdeveloped nations. This study's objectives are to evaluate certain packing media for biological trickling filters (BTFs) and create a simpler model to describe the BTFs' capability for BOD removal.[62]

This study examined BTF with four distinct media, including stone, rubber, polystyrene, and stones, at two temperature ranges: 5–15°C and 25–35°C. At temperature ranges of 5–15 and 25–35°C, respectively, the average removal of COD and BOD was greater than 80% and 90%, respectively.[64] In low temperature range of 5-15°C, the geometric mean of faecal coliforms in BTF was decreased by 4.3, 4.0, 5.8 and 5.4 log10, **respectively, using polystyrene, plastic, rubber, and stone as filter medium.** [65]

Area of Advancement Details	
"A seasonal analysis of *Staphylococcus aureus*, methicillin-resistant S. aureus, and the mecA gene in a municipal wastewater treatment facility"	"Treatment for *Staphylococcal* disorders is under danger due to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), in which the "mecA" gene causes resistance. The objectives were to ascertain how wastewater treatment methods affected "mecA" gene concentrations as well as the evolution of *S. aureus* and MRSA prevalence. Real-time PCR tests were used to examine a municipal wastewater treatment facility for the "mecA" gene, *S. aureus,* and MRSA. "[68] "At eight locations across the facility, water samples were taken on a monthly basis for a year to represent various components of the treatment process. At all sampling sites, the mecA gene and *S. aureus* could be found all year long. MRSA might also be found, although mostly during the first stages of therapy. Through inlet water cultivation, MRSA presence was confirmed. The mecA gene's concentration fluctuated across months and sample locations, but no discernible seasonal change could be seen. The mecA gene content was decreased in most months by the wastewater treatment procedure."[69] "MRSA was discovered for the first time in a municipal activated sludge and trickling filter through molecular techniques and culturing, mostly in the early stages of treatment."[70] "The wastewater treatment process reduces *mec*A gene concentrations, which can partly be explained by removal of biomass."[71]
"Detection of genes associated with antibiotic resistance in biofilms from wastewater, surface water, and drinking water"	"Biofilms from wastewater systems, particularly from secondary of sewage treatment facilities, include high bacterial densities and variety. Surface water and drinking water distribution systems also produce biofilms The majority of research on antibiotic resistance in aquatic environments has been on bulk water and does not account for the condition in biofilms, which are many bacteria's preferred habitat."[72] "In order to determine the prevalence of resistant bacteria and their resistance genes, biofilms from a sewage treatment plant, from surface water, and from drinking water from river bank filtrate were examined."[73] "Determined were the populations of heterotrophic bacteria, *Enterococci, Staphylococci,* *and* *Enterobacteriaceae*. Resistance was assessed using both bacteria grown from biofilms and complete genomic DNA extracted from uncultivated biofilm populations."[74] "By using PCR and Southern blot hybridization, it was possible to find the vancomycin-resistance gene, vanA, from enterococci, the methicillin-resistance gene, mecA, from staphylococci, and the -lactam-resistance gene, ampC, unique to certain *Enterobacteriaceae."*[75]
"Utilising a trickling filter to biotreat nitrogenous compounds released in fishmeal plant exhaust gases"	"Production of fishmeal has significant environmental concerns with odour emissions. In order to explore the biotreatment of low-loads of methylamines and ammonia, which are the principal ingredients of pungent exhaust gases generated by fishmeal processing facilities, laboratory-scale biotrickling filters (BTFs) were infected with microbial consortia obtained from sewage sludge."[76] "An actual fishmeal plant emission including trimethylamine (TMA), dimethylamine (DMA), and monomethylamine (MMA) was tested on a BTF filled with ceramic rings. Following 30 days of operation, the highest elimination capacities (ECs) were 372 mg TMA m3 h1, 5.518 mg DMA m3 h1, and 1.038 mg MMA m3 h1, with maximum removal efficiencies of 92% (TMA), 83% (DMA), and 95% (MMA).[77] "These findings indicated the possibility of odour removal by using BTFs inoculated with a methylotrophic microbial consortium to eliminate volatile amino-compounds." [78]

CONCLUSION

In this research, there has been an opportunity to treat the waste water in a organic way which is eco-friendly, exceptionally in low budget rather than other ways, there has a reuse of waste and grow microorganism. They can form biofilm to treat the wastewater. The applicability can be expanded to sustainable municipal wastewater treatment in rural areas, factories and underdeveloped nations. In secondary wastewater treatment methods, microorganisms are utilised to biologically remove contaminants from wastewater. Both aerobic and anaerobic secondary biological activities need different bacterial populations.

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