Nepal J Biotechnol. 2024 Jul; 12 (1):16-31 Review article DOI: https://doi.org/10.54796/njb.v12i1.318

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal
Journal Homepage: https://nepjb.com/index.php/NJB
ISSN (Online): 2467-9313
ISSN (Print): 2091-1130

Biofilm Reactors: A Potential Alternative to Current Treatment Technology for Wastewater in Kathmandu Valley

Pratap Bikram Shahi[1,2], Sarita Manandhar[3] and Shukra Raj Paudel[1]

[1]Department of Civil Engineering, Pulchowk Campus, Institute of Engineering, Tribhuvan University, Nepal
[2]Aastha Scientific Research Service Pvt. Ltd., Maitidevi, Kathmandu, Nepal
[3]Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Nepal

Received: 06 May 2024; Revised: 20 May 2024; Accepted: 28 May 2024; Published online: 31 Jul 2024

Abstract

Kathmandu Valley faces challenges managing its growing wastewater volume, compounded by the complex composition of unregulated industrial discharges. Releasing untreated wastewater poses a severe risk to public health and the environment. Existing wastewater treatment infrastructure, primarily reliant on conventional activated sludge processes (ASP) struggles to meet growing demands. These systems require substantial land area, are sensitive to influent variations, produce a high volume of sludge, and incur high operational and maintenance costs.

Biofilms, naturally occurring assemblages of microorganisms adherent to surfaces and embedded within an extracellular polymeric matrix (EPS), present a compelling alternative for wastewater treatment due to their diverse pollutant removal capabilities. When implemented as biofilm reactors, they offer distinct advantages, including tolerance to fluctuations in wastewater composition, minimal land requirements, and reduced energy consumption. Notably, microbes residing within a biofilm are capable of biodegradation of persistent materials such as pharmaceuticals, metals, and plastics. Globally, biofilm-mediated wastewater treatment has been implemented successfully, while a knowledge gap remains for the treatment of Kathmandu's wastewater.

This review critically assesses biological wastewater treatment methods, providing insight into: a) suspended growth process with their configuration, application, and limitations, b) wastewater treatment infrastructures of Kathmandu Valley, and c) biofilm process with their configuration, factors influencing biofilm development and performance, application of specific microbial strains for enhanced treatment efficiency, and factors to be considered during implementation. Furthermore, the paper recommends: a) an extensive study of laboratory-scale biofilm reactors evaluating and optimizing their performance for local integration and b) investigating the role of diverse microbial communities to further enhance the treatment plant's operation. By prioritizing research and development towards biofilm technology, Kathmandu Valley can achieve efficient and environmentally friendly wastewater management.

Keywords: Biofilm, Biological Treatment, Kathmandu Valley, Wastewater

✉ Corresponding author, email: srpaudel@ioe.edu.np

Introduction

Wastewater is defined as water that has been impacted by anthropogenic activities leading to deterioration in its physiochemical properties. This effluent incorporates liquid waste discharge that originates from human activities such as domestic use (excreta, urine, cooking, bathing, washing, etc.), commercial and industrial applications (food and paper processing, manufacturing and hospitality management), and agricultural practices (fertilizers, pesticides, and animal husbandry) [1–3]. The detrimental effects of discharging untreated wastewater are multifaceted, encompassing both public health and the environment [4]. Pathogen exposure from untreated wastewater can cause skin and kidney problems, and increase the spread of infectious diseases such as gastrointestinal, typhoid, cholera, and diarrhea [5,6]. Environmental impacts of untreated wastewater disposal range from pollution of surface water and groundwater to adverse effects on biodiversity [7]. Poor wastewater management threatens the extinction of around a million plants and animals [8]. Additionally, the dispersion of untreated wastewater effluent and sludge in terrestrial ecosystems results in the gradual accumulation of persistent organic compounds and toxins in the environment [9]. Furthermore, inadequate wastewater management hinders the overall progress towards the interconnected sustainable development goals (SDGs) [10].

A study by Jones et al., (2021) [11] assessed that only 52% of the global wastewater volume produced annually, estimated at 359.4 x 10^9 m^3, undergoes treatment. Moreover, the same study highlighted North America as having the highest per capita wastewater generation rate, at 209.5 m^3/year, while lower-middle-income economies such as Nepal have a generation rate of 22.5

m^3/capita/year. Nepal's urbanization is on the rise, with a significant portion of the population concentrated in major cities like Kathmandu, Pokhara, Lalitpur, Bhaktapur, and Birgunj [12]. Driven by a continuous influx of people migrating from rural regions to cities, this migration is primarily motivated by the pursuit of improved employment opportunities, educational advancement, and access to various amenities [13,14]. This trend has intensified the wastewater management complex and placed significant pressure on the limited water resources within these urban centers [15,16].

Kathmandu Valley exhibits the most extensive urbanization with the largest built-up area (37.7%) and the maximum gain in built-up area of 368.08% increase within the past three decades [17]. Despite a reported 70% sewerage network coverage in the valley, the sole operational Guheshowri Wastewater Treatment Plant can only process 12% of the total wastewater generated [18]. Furthermore, projections indicate a significant rise in wastewater generation within the valley, reaching 350 million liters per day (MLD) by 2030, meanwhile, only an estimated 44% of this volume can be treated with existing and proposed wastewater treatment infrastructures [19]. A study by Koju et al., (2022) [20] revealed that an estimated 228 industrial facilities within the valley directly discharge untreated effluents, which typically contain high concentrations of nitrate, total suspended solids, calcium hardness, and heavy metals such as iron (Fe), arsenic (As), zinc (Zn), and lead (Pb) into waterways or sewer networks. Some of these heavy metals are toxic and can be carcinogenic or teratogenic potentially causing nervous system damage, organ dysfunction, or impaired development and growth [21].

Wastewater treatment utilizes various effective technologies for the removal of pollutants from wastewater. These encompass biological processes, such as the ASP and waste stabilization ponds, and physicochemical methods, including membrane technologies and advanced oxidation processes [22]. Coagulation-flocculation is a common and effective physicochemical process for removing turbidity, organic matter, and suspended solids from wastewater [23,24]. Metal salts when applied as coagulants are also capable of inactivating bacteria [25]. Recent research has been focused on the application of natural coagulants as an environmentally friendly alternative to chemical coagulants [26]. For instance, a study by Boulaadjoul et al., (2018) [27], showed that *Moringa oleifera* seed when used as a natural coagulant for the treatment of effluent of the paper mill industry achieved 97.3% removal of chemical oxygen demand (COD), compared to 92.7% with aluminum sulfate (alum). However, the drawbacks of using coagulants are [26]: a) chemical coagulants have high and toxic sludge generation and b) natural coagulants can release organic matter into treated effluent. Membrane technology with its modularity applies membrane pores for pollutant separation and can also handle emerging pollutants and is either driven by pressure or osmotically [28,29]. A study by Gebru & Das, (2018) [30] applied modified cellulose acetate ultrafiltration with TiO_2 nanoparticles that demonstrated excellent removal of chromium (VI) ions up to 99.8%. However, the major drawbacks of membrane technology are membrane fouling and maintenance of membrane modules [31]. Advanced oxidation processes are chemical treatments suitable for wastewater with toxic or non-biodegradable compounds [32]. In this process oxidizing radical groups such as hydroxyl radicals are generated that oxidize and mineralize organic compounds into H_2O and CO_2 [33,34]. Research conducted by Doltade et al., (2022) [35] achieved 91% COD removal from dye wastewater using (hydrogen peroxide) H_2O_2 and ozone. The disadvantages of using an advanced oxidation process for the treatment of wastewater are: a) power outage that can inhibit effective system operation thus requiring expensive operational and maintenance equipment and b) potential unregulated by-products that may be formed in the treated effluent.

The overall objectives of biological wastewater treatment are the transformation of biodegradable pollutants into acceptable end products, nutrient removal, immobilization of solids into the biofilm matrix or biological floc, and elimination of trace constituents and compounds [36]. While biofilms matrix host surface-adherent communities encased within a self-secreted biopolymer matrix, biological flocs are suspended aggregates of bacteria held together by a similar EPS [37,38]. Microbes achieve the stabilization of organics by two distinct pathways [39]: a) respiration (oxidation of substrate with release of energy) and b) synthesis (utilization of the energy produced by respiration and remaining substrate for production of new protoplasm and maintenance of the cell). Microbes can be classified as aerobic, anaerobic, and fermentative based on their preferred catabolic pathways for energy production [40]. Aerobic biodegradation utilizes O_2 as the terminal electron acceptor (TEA) during the catabolization of organic compounds [41]. This results in an increase in microbial population, and CO_2, H_2O, and other compounds as byproducts [42]. Conversely, anaerobic biodegradation employs CO_2, NO_3^-, SO_4^{2-}, organic molecules, and some oxidized metal ions such as Mn^{4+} and Fe^{2+} as alternative TEA [43–46]. In the fermentative process, microbes utilize the organic molecules as electron acceptors [46]. Finally, biological systems can be classified based on how microorganisms are retained within the treatment reactors as suspended growth processes (planktonic) and attached growth processes (biofilm) [47].

While suspended growth systems such as ASP are widely used in wastewater treatment, they have high operation and maintenance (O&M) and design costs [48]. Biofilm reactors offer a promising alternative due to their compact design, efficient pollutant removal capabilities, and lower operational demands [49], potentially addressing these limitations. Biofilms are communities of microbial cells attached to a surface and enclosed by an EPS [50]. These biofilms can harbor diverse microbial populations, either homogeneous or heterogeneous species composition [51]. Bioremediation of contaminates through the application of biofilm is an environmentally friendly and cost-effective approach [52]. Through metabolic processes such as biomineralization, biosorption and bioaccumulation microbes within the biofilm can remove even slow degradable pollutants from wastewater [53]. Globally, the treatment of wastewater has been carried out using biofilm in the form of Tricking Filters (TFs), Rotating Biological Contractors (RBCs), Moving Bed Biofilm Rectors (MBBRs), and others [54].

A 2017 study by Gurung et al., (2017) [55] identified approximately 26 constructed wetlands (CWLs) used for secondary wastewater treatment in Nepal. These systems function on a symbiotic relation between the macrophyte and microbes [56]. Macrophytes encompass a wide range of plant life, including vascular plants, bryophytes, green macroalgae, and charophytes that thrive entirely or partially submerged in aquatic environments [57]. CWLs promote biofilm growth on submerged plant roots and within porous root beds, facilitating the removal of organic compounds [58–60]. However, the current CWL design in Nepal primarily focuses on plant selection [61–63]. This highlights a substantial knowledge gap regarding the targeted application of biofilm technology for wastewater treatment within the Kathmandu Valley. Coupled with limited land availability for construction and expansion of conventional ASP further emphasizes the need for research and development of alternative wastewater treatment technologies, particularly those utilizing biofilm processes.

The review paper aims to comprehensively analyze published research on biofilm reactor technology for wastewater treatment providing insight into their various configuration, advantages, factors influencing their performance, and microbial dynamics. Furthermore, the review assesses the advantages and disadvantages of suspended growth biological process and the current state and proposed advancements in wastewater treatment infrastructure within the Kathmandu Valley. By undertaking a meticulous examination of existing literature, the paper aims to identify knowledge gaps that hinder the optimization of biofilm reactor performance for Kathmandu's wastewater treatment. This review will serve as a foundational resource for future research endeavors by outlining key areas for investigation. These areas encompass the application of specific microbial strains for enhanced treatment efficiency, the evaluation of microbial genomic profiles within reactors operated over extended durations, and the exploration of unconventional metabolic pathways employed by the microbial consortium for improved pollutant removal.

Review Methodology:

A comprehensive review of recent peer-reviewed studies was undertaken to evaluate the potential application of biofilm reactor technology for wastewater treatment within the Kathmandu Valley.

Search strategy

Academic databases such as ACS, Google Scholar, Scopus, PubMed, and others were searched using keywords such as "biofilm reactors," "wastewater treatment," "Kathmandu Valley," and relevant terms. The literature search focused on recent peer-reviewed articles published in English. The emphasis was on studies published after 2020, to ensure the most current information.

Inclusion/Exclusion criteria

The search specifically targeted studies investigating biofilm reactor technology for wastewater treatment applications. Included studies explored the mechanisms by which these reactors remove pollutants and factors impacting their performance. Conversely, studies solely focused on individual contaminants or applications outside of wastewater treatment were mostly excluded.

Selection process

Following the initial screening of titles and abstracts, full-text articles of potentially relevant studies were retrieved for a more in-depth evaluation based on the predetermined inclusion and exclusion criteria.

Table 1. Application of suspended growth process based treatment systems.

Treatment Technology	Wastewater Type	Target Pollutants	Removal Efficiency	References
Activated Sludge Process	Pickled-vegetable plant sewage	COD	90%	[73]
Activated Sludge Process (co-treatment)	Dairy (cheese and milk) and domestic wastewater	Carbon and NH_4-N	Carbon (both types): 87%, NH4-N (cheese): 95%, NH_4-N (milk): 75%	[74]
Sequencing Batch Reactor (anammox)	Real domestic wastewater	TN	TN (anammox): 89%, TN (denitrification): 11%	[75]
Sequencing Batch Reactor	Synthetic domestic wastewater	NO_3-N, COD, NH_3-N	NO_3-N: 78%, COD: 93%, NH_3-N: 83%	[76]
Extended Aeration Activated Sludge System	Pulp and paper industry wastewater	Ammonia	Achieved standard limit (10 mg/L) at 24 hr HRT	[77]
Waste Stabilization Pond	Wastewater with tetracycline	COD, Tetracycline	COD: 80 ± 4%, Tetracycline: 99% (7 days HRT)	[78]
Waste Stabilization Pond	Household swine wastewater	TN, COD, TP	TN: 84%, COD: 74%, TP: 84%	[79]
Membrane Bioreactor (PVDF flat sheet)	Municipal wastewater	COD	89%	[80]
Membrane Bioreactor (submerged anaerobic)	Domestic wastewater	COD	89% (6-12 hr HRT)	[81]
Membrane Bioreactor (hollow fiber)	Municipal wastewater	COD	92.8% (6-12 hr HRT)	[82]

Where, NH_3-N = Ammonia-Nitrogen; NH_4-N = Ammonium-Nitrogen; TP = Total Phosphate; TN= Total Nitrogen

Data analysis and synthesis

A narrative synthesis approach was employed to analyze and synthesize the findings from the selected studies. This method involved the exploration of recurring themes, patterns, and any potential inconsistencies within the research. The analysis specifically focused on the application of biofilm reactor technology for wastewater treatment, particularly in the context of municipal wastewater treatment.

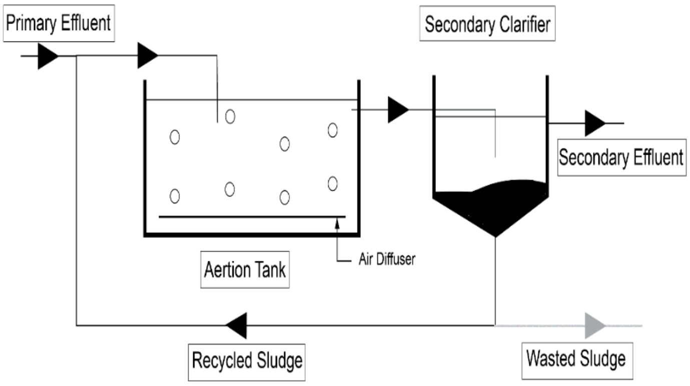

Figure 1. Schematic flow diagram of Activated Sludge Process (ASP) [Modified diagram of [70]]

Suspended Growth Wastewater Treatment Technologies

In a suspended growth system, aggregates (flocs) of microorganisms responsible for treatment grow in contact with the liquid that is to be treated [64]. These microbes without attachment to any surfaces, grow in the planktonic state within the bulk medium [47]. Depending on the wastewater type, the process can be aerobic for the treatment of municipal and industrial wastewater, or anaerobic for the treatment of organic sludge and concentrated industrial wastewater [65].

In conventional aerobic suspended growth systems such as ASP, air agitation within the aeration tank enhances the development of microbial flocs, known as activated sludge (AS) [66]. Here, the primary effluent is mixed with settled solids from the secondary clarifier before introducing into the aeration tank (**Figure. 1**). Continuous aeration is provided through diffusers positioned at the tank's bottom [67]. Sludge recirculation ensures a resident microbial population within the aeration tank, facilitating efficient organic compound oxidation of the incoming wastewater [68]. Higher influent organic load necessitates a proportional increase in the aeration tank's microbial population, achieved by adjusting the sludge recycling rate based on secondary clarifier sludge concentration and existing microbial density in the aeration tank [69].

The characteristics of AS flocs developed in the APS are highly influenced by operational process parameters such as Hydraulic Retention Time (HRT), nutrient availability, Recycling Ratio, Carbon/Nitrogen ratio, and solid retention time [71,72]. Many studies have successfully demonstrated the effective treatment of wastewater with varying characteristics using conventional ASP and its modifications (**Table 1**).

One of the modifications is the Sequencing Batch Reactor (SBR), which treats wastewater in batches within a single aeration tank, eliminating the requirement of separate clarifier [83]. The operation of SBR is as follows [84]: a) Wastewater is added to the tank containing biomass, b) The biomass and wastewater mix for a set time, allowing microbes to consume organic matter, c) Aeration and mixing may or may not be carried out during this stage, d) The contents within the tank is allowed to rest/settle for efficient separation of solids and liquid, e) Effluent and sludge removal from tank, and f) The process restarts with the addition of a new batch of wastewater.

Waste Stabilizing Ponds (WSPs) a type of suspended growth process, offer the utilization of natural processes for wastewater treatment [85]. In this system, bacteria breakdown complex organic matter into CO_2 and simpler compounds which algae then uptake through photosynthesis providing oxygen essential for the sustenance of aerobic bacteria [86]. The WSPs can be divided into three distinct ponds (**Figure 2a**) based on the operational conditions namely: 1) Maturation/Aerobic pond, 2) Facultative pond and, 3) Anaerobic pond [87].

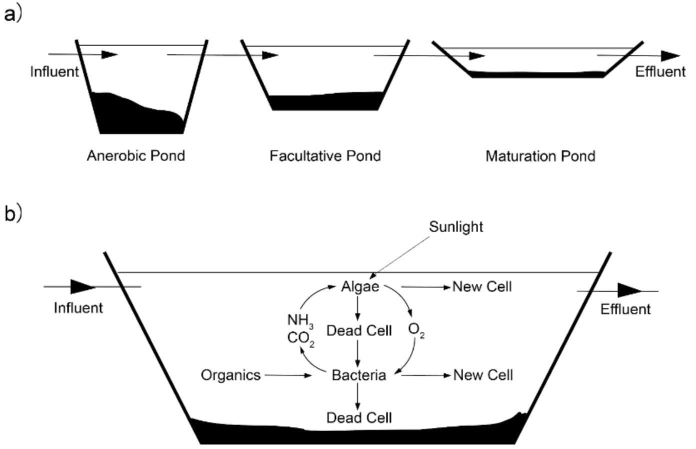

Figure 2. Schematic diagram: a) Configuration of Waste Stabilization Ponds and b) Mechanism of treatment in a Facultative Pond [Modified diagram of [87,88]]

The majority of Biological Oxygen Demand (BOD) removal from wastewater occurs in the facultative or anaerobic pond whose effluent is then fed to the maturation pond for pathogen elimination [89]. A facultative pond (**Figure 2b**) with lesser depth than an anaerobic pond, requires a larger land area and requires ample sunlight for optimal function [90]. Meanwhile, the maturation pond presents itself as a low-cost alternative for pathogen removal (disinfection) and can also be integrated with other wastewater treatment technologies [91]. Recently, studies have explored modifications to WSPs that enhance algal biomass to potentially improve treatment efficiency (**Table 1**).

Membrane bioreactors (MBRs) combine a conventional ASP system with membrane filtration technology (Fig. 3) [92]. The degradation of pollutants occurs in the bioreactor i.e. ASP, while the membrane filter separates the microorganism from the treated water [93].

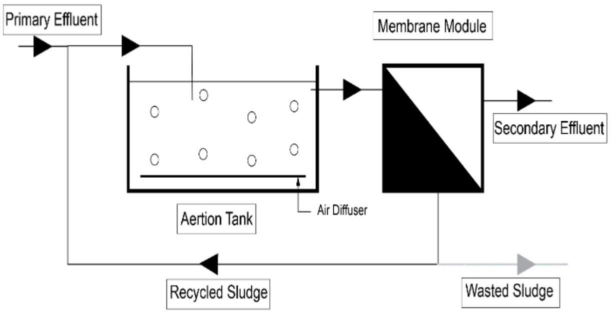

Figure 3. Schematic flow diagram of Membrane Bioreactor (MBR) [Modified diagram of [94]]

Since there is no requirement for a secondary clarifier after the aeration tank, this allows for a smaller-sized bioreactor compared to conventional ASP [95]. The effectiveness of MBRs in treating wastewater varies with the type of membrane material used, as shown in **Table 1**.

Despite their effectiveness in treating diverse wastewater, suspended growth systems still face certain limitations as presented in **Table 2**.

Table 2. Limitations of suspended growth process based treatment systems.

Treatment Unit	Limitations	Ref
Activated Sludge Process	Requires longer HRT; Highly sensitive towards variation in influent wastewater flow and characteristics and unexpected modification of operating conditions; Filamentous bulking of AS; Lower sludge retention time; Higher Operational cost and requirement of continuous supervision.	[96–99]
Waste Stabilizing Ponds	Requires large land area for construction hence a higher capital cost; Long retention time; Relatively ineffective in colder regions; Release of biogas; Not suitable for nutrient removal; Provides breeding ground for mosquitoes;	[100]
Sequencing Batch Reactor	More sophisticated control system than conventional ASP; Capable of treating relatively smaller volume of wastewater;	[83,101,102]

Membrane Bioreactor	Low pathogen removal; Membrane fouling and high maintenance cost; Dewatering of sludge more difficult due to sludge flocculation;	[93,103]

These limitations of suspended growth biological systems present a significant economic and sustainability challenge for developing countries like Nepal. Similarly, these systems necessitate a dedicated and well-designed sludge management strategy for optimal operation.

Wastewater Treatment Infrastructure in Kathmandu Valley

Before 2000 A.D., the Kathmandu Valley operated five prominent (**Table 3**) decentralized wastewater treatment plants (DEWATS) [104]. However, most of these facilities became non-functional due to insufficient operation and management practices, malfunctions in flow control systems, and recurring power outages [19].

Table 3: Decentralized treatment plants in Nepal and their service area prior to 2000 A.D. [104]

Location	Catchment Served
Dhobighat	Kathmandu & Lalitpur
Guheshwori	Gokarna & Chabahil
Hanumanghat	North-east Bhaktapur
Kodku	East Lalitpur
Sallaghari	North & South Bhaktapur

Several of these wastewater treatment systems with conventional ASP are currently undergoing rehabilitation and upgradation, meanwhile, two Moving Bed Biofilm Reactors (MBBRs) are proposed as DEWATS at Hanumanghat (1 MLD) and Gokarna (3 MLD) which are currently under construction [105].

Employing suspended growth biological treatment systems, such as conventional ASP, offers a proficient approach to eliminating organic pollutants from wastewater, achieving removal rates of 84-86% COD [106]. Although conventional ASP effectively treats wastewater, their limited nutrient removal necessitates their modification by extending the mean cell residence time (MCRT) and HRT or incorporating an additional tertiary treatment for complete nitrate and phosphate elimination [36,107]. Additionally, the performance of these systems is highly susceptible to operational parameters like temperature and HRT [108].

The Guheshowri Wastewater Treatment Plant (WWTP) with conventional ASP as the biological treatment unit and recently upgraded to improve the effluent wastewater quality, currently boasts a treatment capacity of 32.4 Million Liters per Day (MLD) [105]. This modernization incorporated treatment units like primary sedimentation tanks, chlorination for disinfection at the beginning of serpentine effluent detention chamber, dechlorination to neutralize chlorine residuals before effluent discharge, and disc filters (**Figure 4a**) for enhanced removal of solids along the primary flow line (Wastewater Treatment) [109].

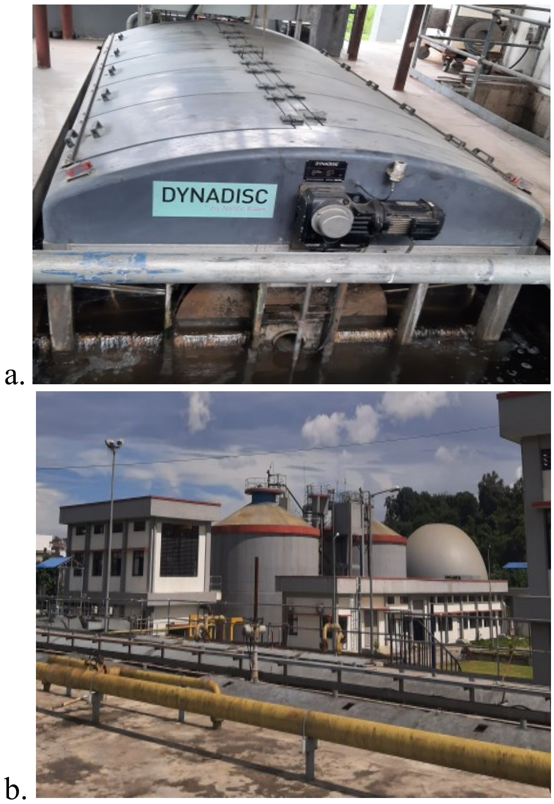

Figure 4. Treatment units added to Guheshowri WWTP after modernization: a) Disc Filter and b) Anaerobic Digesters

The secondary flow line (Sludge Treatment) also benefited from advancements, with the addition of sludge thickeners, dewatering units, anaerobic digesters (**Figure 4b**), and biogas collection units to capture the generated biogas providing a sustainable energy source for the treatment plant's daily operations [109]. However, the conventional treatment plant's increased dependency on a high degree of mechanization, inherent uncertainties, natural conditions, and influent variation introduces uncertainty that leads to discrepancies in operational cost, environmental risks, and effluent quality [19,110].

Biofilm Technology

Wastewater's dynamic physicochemical profile (chemical composition, pH, temperature, turbidity) fosters diverse microbial communities. These communities adapt by forming complex biofilms, which are adherent assemblages of microorganisms. Biofilms provide protection and communication channels for the microbial colonies, playing a crucial role in nutrient capture and consumption [111,112]. The life cycle of biofilm (**Figure 5**) begins with [52]: a) Attachment: adhesion of free-floating microbes (planktonic) to the carrier surface, b)

Development and Maturity: recruitment of planktonic microbes, reproduction, and increase in the cell density, c) Death and dispersion: dispersion through halt in production of EPS or sloughing caused by shear or hydrodynamic force.

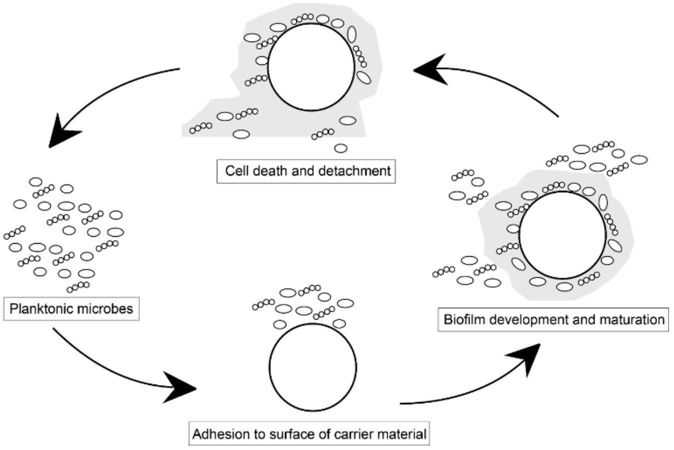

Figure 5. Diagrammatic View of Life cycle of Biofilm Mechanism [Modified diagram of [113]]

EPS enhances the biofilm's mechanical and chemical stability, functions as a bioadsorbent, effectively capturing and concentrating metals within the biofilm, and provides a protective barrier, shielding the biofilm from various environmental stresses [47,114]. Within a biofilm, diffusion is the predominant transport process of substrates and electron acceptors, resulting in spatial variations in substrate concentration throughout the biofilm structure [115,116]. Biofilms harbor specialized microbes deeper within their structure, offering protection and promoting efficient removal of micropollutants [117].

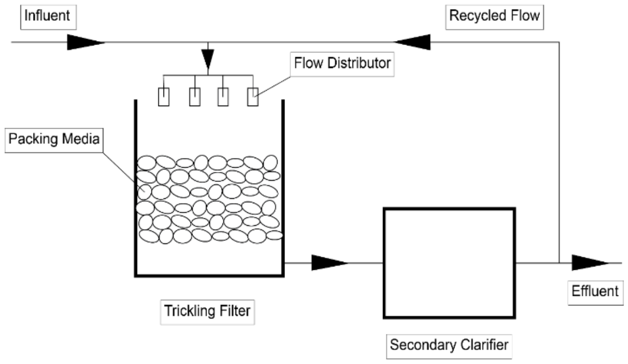

Figure 6. Schematic diagram of Trickling Filter [Modified diagram of [122]]

Wastewater treatment using biofilm

Biofilm reactors are a biological wastewater treatment system designed to leverage the metabolic capabilities of microbial communities by fostering their growth as biofilms on surfaces within the reactor [118]. Biofilm can be engineered into reactors namely Trickling filter (TF), Submerged Fixed Bed Biofilm Reactor (SFBBR), Moving bed biofilm reactor (MBBR), Biological Aerated Filter (BAF), and Rotating Biological Contractor (RBC) [54]. A TF utilizes large packing media (specific biofilm surface area $a_F = 50$ to 200 m^2/m^3) typically plastics or rocks, over which wastewater tricks downwards from a distribution system [117,119]. TFs have demonstrated excellent oxidation of carbon and combined oxidation of carbon and nitrification [54]. Total suspended solid is produced in the effluent treated through TF (**Figure 6**) hence, requiring a circular or rectangular secondary clarifier [52,120]. The recirculation rate generally varies from 0.5 to 4 times the influent flow but can reach up to 10 times for strong industrial wastewater [121].

An SFBBR (**Figure 7**) is usually constructed with small-sized (0.7-8.0mm) granular media providing a specific surface area of 1,000-3,600 m^2/m^3 and operated in a fully submerged condition [117]. These relatively new types of biofilm reactors are primarily used for the treatment of municipal and industrial wastewater [123]. In these reactors, the use of a smaller filter medium allows the combination of biological conversion processes with depth filtration, retaining suspended solids, and eliminating the need for downstream treatment for solids removal while the excess biofilm is usually removed through regular backwashing of the filter [124].

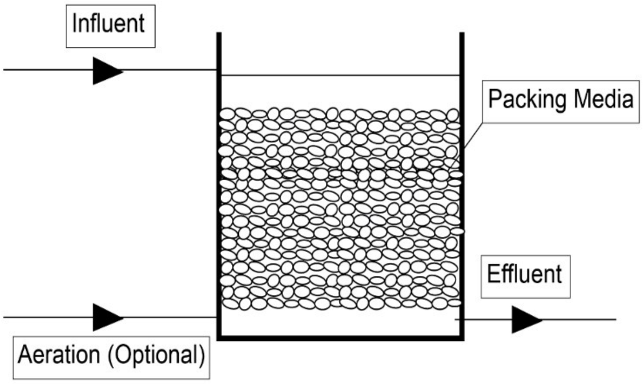

Figure 7. Schematic diagram of Submerged Fixed Bed Biofilm Reactor [Modified diagram of [122]]

Meanwhile, the MBBR system utilizes polyethylene carriers that have a high surface area for the establishment of adherent biomass, while the remaining microbial population exists in a suspended state within the liquid phase [125]. The support medium is kept in suspension through mechanical mixing (**Figure 8a**) or aeration (**Figure 8b**) [126]. A comparative investigation revealed that an MBBR system exhibited a twofold increase in effluent treatment capacity compared to an activated sludge system while maintaining equivalent pollutant removal efficiency [127]. The advantage of MBBR is its high rate of pollutant removal coupled with stability and compactness [123]. The media used in MBBR provides a protective surface for biofilm development thus being able to achieve nitrification even at very low temperatures [128]. Similarly, the low hydraulic headloss in the reactor makes it suitable to be implemented in specific treatment steps such as carbon removal, nitrification, and pre-denitrification [117].

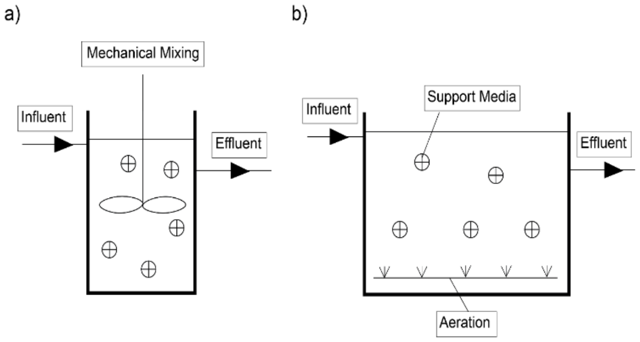

Figure 8. Schematic diagram of Moving Bed Biofilm Reactor: a) Anaerobic/Anoxic reactor with mechanical mixing and b) Aerobic reactor with air diffuser [Modified diagram of [129]]

Table 4: Effectiveness of different biofilm technologies for treatment of different wastewaters.

Treatment Technology	Application	Key Results	Ref
Tricking Filter (Maize cob and Date Palm Fiber)	Municipal Wastewater treatment	Increased pollutant removal by Maize cob (8-15%) higher compared to Date Palm Fiber	[130]
Tricking Filter (Expanded Polystyrene Media)	Wastewater treatment	Achieved 80.75% COD removal	[131]
Up-flow Fixed Bed (Scoria Packing)	Municipal Wastewater Treatment	Improved ammonia & total nitrogen removal with anaerobic/aerobic cycles	[132]
Anaerobic Fixed Bed Biofilm Reactor	Domestic Wastewater Treatment	Higher COD removal (2.9 per hr) & biomethane production (154N-mLCH_4 per gm COD removed) compared to single-phase system	[133]
Moving Bed Biofilm Reactor (Zinc-Doped Carriers)	Moving Bed Biofilm Reactor	Achieved 93% BOD, 80% NH_4-N, and 70% COD removal	[134]
Moving Bed Biofilm Reactor (Partial Nitritation-Anammox)	Municipal Wastewater Treatment	Achieved nitrogen removal rate of 0.66 g N m^2/d	[135]

The biofilm reactors applied in different configurations have proven their effectiveness in the removal of pollutants from wastewater as demonstrated in **Table 4**.

Biofilm composition, particularly the dominant microbial taxa, dictates its classification and allows for targeted inoculation of wastewater treatment plants with specific microbial communities to defined pollutants, enhancing overall treatment efficiency [52]. Multiple research (**Table 5**) have investigated the efficacy of immobilized algae in treating municipal wastewater. Beyond the primary mechanism of nutrient (phosphorus and nitrogen) removal via cellular uptake, microalgae possess the additional capability to sequester excess nutrients through a process known as luxury uptake [136].

In a 2020 study, Chen et al., (2021) [137], demonstrated the efficacy of a Rotating Algal Reactor (RAR) for removing pharmaceutical and personal care products (PPCPs) from wastewater. This system achieved a high removal efficiency, ranging from 70% to 100%, without significantly compromising the nutrient removal capacity of the algal biofilm reactor. Furthermore, algal biofilm benefits include potential integration for biodiesel production via lipid accumulation within the algae, utilization of biomass as a raw material for bioplastic production, and even the exploration of the biomass as a feedstock for animals [138,139].

Paralleling the application of algae, research has identified various bacterial strains with the potential to augment wastewater treatment efficiency. A study by Gao et al., (2020) [144] identified *Simplicispira*, *Diaphorobacter*, *Hydrogenophaga*, *Pseudoxanthmonas*, and *Stenotrophomonas* as dominant bacteria in an up-flow denitrification reactor, achieving over 80% for COD and 97% nitrate removal from wastewater. Isolating a novel *Pseudomonas* sp. (Y39-6), Zhang et al., (2021) [145] demonstrated its potential for wastewater treatment through aerobic-autotrophic nitrate removal in a moving bed biofilm reactor, achieving a 24.83% removal efficiency under low carbon to nitrogen ratio (C/N) < 1. Similarly, Khosravi et al., (2020) [146] explored using *Escherichia coli* biofilm immobilized on zeolite to remove Zn and copper from solution, achieving removal efficiencies of 57.35% for Zn and 54.61% for copper after a 10-day contact time. Research by Begum & Radha, (2016) [147] achieved high efficiency in treating industrial phenol-rich effluent using *Pseudomonas fluorescens* in an inverse fluidized bed biofilm reactor, removing 98.70% of COD and 100% of phenol. EPS isolated from *Acinetobacter junii* BB1A biofilm exhibited significant flocculating activity (94% at 30 mg/L) against kaolin suspension, highlighting the crucial role of EPS-associated amide groups in this process [148].

Table 5. Nutrient removal efficiencies using algal biofilm-based treatment systems.

Parameter	Influent mg/L	Effluent mg/L	Percent Removal	Type	Ref
TP	2.1	1.6	23.8	Municipality	[140]
TN	91.1	19.1	79.0	Municipality	[141]
NH$_4$-N	5.4	0.2	96.3	Municipality	[142]
NO$_3$-N	5.57	2.2	60.5	Municipality	[143]

Where, TP = Total Phosphorous; TN = Total Nitrogen

Beyond the individual benefits of algae and bacteria, a symbiotic consortium of these microorganisms can achieve even greater wastewater treatment efficiency. The study by Tang et al., (2018) [149] showed a synergistic effect in wastewater treatment, where adding algae to a sequencing batch biofilm reactor significantly improved nutrient removal, achieving a 27.3% increase in total nitrogen removal and a 65.8% increase in total phosphorus removal compared to domestic wastewater treatment without algae. Further emphasizing the collaborative nature of this approach, Amini et al., (2020) [150] identified a 5:1 ratio of algae to activated sludge as optimal for nutrient removal from municipal wastewater using algal-bacterial photo-bioreactors. While comparing trickling filters containing an algae-bacterial consortium to a reactor with only bacteria, Katam et al., (2020) [151], found that the algae-bacterial reactor achieved significantly higher removal efficiencies for caffeine (up to 96%) and alkylbenzene sulfonate (up to 99%) from wastewater.

These biofilm reactors offer a compelling alternative for wastewater treatment plants due to their multitude of advantages. These reactors excel at simultaneously removing both organic pollutants and nutrients from wastewater [141,152]. Additionally, biofilm reactors boast impressive adaptability, allowing them to function effectively even when operational conditions fluctuate within the treatment plant [49]. Furthermore, these systems generate significantly less sludge compared to suspended growth processes [153], simplifying waste management. The user-friendly operational nature of biofilm reactors further enhances their appeal, making them a sustainable and efficient solution for wastewater treatment facilities [154].

Factor affecting the formation of biofilm

Biofilm reactors rely on fostering a healthy community of microbes on a carrier surface to optimize wastewater treatment performance. This section explores how various factors, such as pH, surface characteristics of the carrier material, and flow regime, can significantly influence biofilm formation and development. Optimizing these parameters is crucial for promoting and sustaining biofilm, which ultimately leads to improved treatment efficiency [155,156].

1 Surface Topography

Several surface properties influence biofilm formation. Studies have shown that increasing the hydrophobicity and roughness of a surface enhances biofilm attachment and growth [157,158]. Additionally, porous surfaces provide a favorable microenvironment for microbial attachment and proliferation, offering a protective space for biofilm development [159]. Research by Lu et al., (2020) [160] found that decreasing surface roughness on ceramics from sub-micron to nano-scale increases hydrophilicity, hindering *Staphylococcus aureus* adhesion. A study by Hsieh & Chien, (2023) [161] investigated biomimetic surfaces for bacterial adhesion of *Escherichia coli* and *Staphylococcus aureus*, revealing that smaller features hampered biofilm formation, along with hysteresis angle playing the key role in influencing bacterial attachment. Another research by Zhang et al., (2020) [162] revealed that rougher surfaces with more stagnant zones and asperities enhanced algal adhesion by promoting initial interception, retention, and strengthening the attachment force. Similarly, Polyethylene Terephthalate (PET) threads surface engineered using chromic acid which created new grooves and decreased the contact angle, resulting in an increased rate of microalgae (*Scenedesmus dimorphus*) attachment [163].

2 Temperature and pH

Temperature's impact on biofilm formation is species-specific, but increased cell growth at any temperature generally leads to more EPS production, which enhances biofilm development and provides protection [164,165]. The presence and response rate of enzymes and the features outside and inside cells are influenced by environmental temperatures [166,167]. Gram-positive bacteria exhibit a narrower optimal temperature range (30-37°C) for biofilm formation compared to the broader range tolerated by Gram-negative bacteria (4-50°C) [168]. The research conducted by Morimatsu et al., (2012) [169] found that when nutrients are abundant *Pseudomonas putida* biofilms detach at high temperatures while remaining stable at lower temperatures. A study by Li et al., (2022) [170] reported that colder temperatures (4°C) triggered increased production of specific polysaccharides (with $C=O$ and $O=C\text{-}O$ functional groups), leading to denser and more resistant biofilms. The optimal temperature for development of algal species is between 20°C and 30°C [171]. In another study, Gonzalez-Camejo et al., (2019) [172] reported that microalgae in a mixed community with bacteria completely ceased activity at temperatures exceeding 30°C.

The formation and development of biofilms are highly sensitive to environmental factors, with pH playing a critical role. Generally, the pH of wastewater falls within the range of 6.5 to 8.5, with most values falling above neutrality (pH = 7) [173]. Most bacterial species exhibit peak production of polysaccharides, essential components of biofilms, at a neutral pH of approximately 7 [174]. The EPS matrix surrounding a biofilm acts as a protective shield, enhancing its tolerance to varying pH levels compared to planktonic cells [175,176]. A study by Zmantar et al., (2010) [177] reported significantly reduced biofilm formation of *Staphylococcus aureus* at both acidic (pH = 3) and alkaline (pH = 12) conditions compared to a neutral pH. The research by Li et al., (2021) [178] found that pH significantly affects the bacterial community composition in a microbial fuel cell, with higher pH leading to increased power output. At temperatures of 37°C and pH 7.0 strong biofilm of *Salmonella enterica* was developed on food and food contacted surfaces [179]. Microalgae generally exhibit a negative surface charge across a range of pH values [180]. Research by Z. Zhao et al., (2021) [181] achieved optimal microalgae biofilm growth at a neutral pH (7) due to a positive membrane charge, while a higher pH (10) with a negative charge facilitated harvesting.

3 Velocity, turbulence and hydrodynamics

High flow velocity in wastewater treatment systems can affect biofilm growth by thinning the boundary layer near the surface, exposing cells to turbulence [52]. Hydrodynamic conditions can affect EPS production, its overall size and density, growth rate, structure, and even the metabolic activity of the microbes living within it [182]. A study conducted by Khu et al., (2023) [183], found that biofilm communities exposed to increasing flow velocities (up to 0.49 m/s) exhibited greater diversity and stress resistance, but this beneficial effect diminished at higher velocities. It is suggested that high fluid shear forces caused by faster flow can strengthen an initial bacterial attachment to surfaces by promoting nutrient access [184,185]. A study by Chang et al., (2020) [186] revealed that *Bacillus* sp. grown under low shear formed loose and tower-shaped biofilms limiting mass transfer, while higher shear yielded denser and smoother biofilms with increased EPS production.

Factors to be considered during implementation of biofilm technology

Biofilm not only facilitates mutualism but also competition among microbes vying for limited resources [187]. *Pseudomonas* spp. while applied for treating wastewater with low biodegradable organics might lead to an increase in NH_3 in the effluent as the bacteria are capable of utilizing amino acid as alternative carbon sources which release NH_3 as a byproduct [188,189]. Sudden high ammonia loading can lead to ammonia toxicity, causing nitrogen crashes and leading to fluctuations in effluent NH_3 levels [190,191]. Similarly, the amount of carbon available in a biofilm reactor impacts the rate of denitrification process [192]. In a microalgae-bacterial system, an increase in bacterial population leads to a decrease in nitrate removal rate [193]. Despite the symbiotic relationship between algae and bacteria in these systems, competition for limited nutrients like phosphorus and nitrogen can hinder wastewater treatment performance [194]. At a low dissolved oxygen concentration in wastewater, the removal of nitrogen occurs through unconventional pathways within the biofilm [195]. For the treatment of wastewater with heavy metals using biofilm, the type of donor atoms (Nitrogen, Oxygen, and Sulfur) present in the binding site of the matrix influences the preferred species of heavy metals to be removed [196]. Implementation of biofilm technologies without consideration of these key factors can cause treatment performance to diverge from expected removal trends, leading to a decline in system effectiveness.

Advantages of biofilm reactors

While multiple wastewater treatment technologies exist with their advantages as stated in sections above. The advantage of application of biofilm over other wastewater technologies is summarized in the **Table 6** below: Due to their advantages over conventional methods, biofilm reactors present themselves as a promising alternative for wastewater treatment within Kathmandu Valley.

Conclusions and Future Perspectives

Due to Kathmandu Valley's rapid urbanization, the land available for construction and expansion of wastewater treatment is becoming scarce. This challenge is compounded by the increasing volume of wastewater, whose composition is further complicated by unregulated industrial discharges. Conventional suspended growth process requires extensive areas to

Table 6. Advantage of Biofilm for wastewater treatment over other treatment technologies

Treatment Technologies	Disadvantages of the technologies	Advantage of Biofilm
Activated Sludge Process	- Highly sensitive to influent variations and unexpected operational changes	- Due to the functional diversity of their microbial communities, biofilm systems exhibit enhanced resilience to fluctuations in wastewater composition, promoting a more stable treatment process.
Waste Stabilization Ponds (WSPs)	- Requires large land area, leading to higher capital costs - Relatively ineffective in colder regions	- Biofilm reactors are often smaller and more compact. - Some biofilm systems can operate efficiently at lower temperatures.
Sequencing Batch Reactor (SBR)	- Suitable for treating smaller wastewater volumes	-Biofilm systems can be designed for various treatment capacities.
Coagulation & Flocculation	- Requires addition of chemicals (coagulants & flocculants) - May generate residual solids requiring disposal - Ineffective for removing soluble organics	-The EPS of biofilm have flocculating properties.
Membrane Technologies	- High energy consumption for pressure-driven processes	-Biofilm such as TFs rely on gravity flow eliminating the need for high-energy pumps required in pressure-driven membrane systems.
Advanced Oxidation Processes (AOPs)	- Potential for formation of harmful byproducts - High energy consumption	- Biofilm are potentially capable of biologically degrading some persistent compounds and harmful byproducts.

handle this rising volume of wastewater. Furthermore, these highly mechanized systems are sensitive to variations in influent flow and operational parameters, demanding constant monitoring to ensure proper functioning. While biofilm-mediated wastewater treatment is a well-established technology globally, Nepal's wastewater management has only recently begun incorporating this technology. Biofilm treatment involves attaching free-floating microbes to surfaces, creating a diverse community that works together to remove pollutants from wastewater. These technologies emerge as a promising alternative, offering a sustainable solution for wastewater treatment with minimal operational cost and maintenance energy requirements. To effectively implement large-scale biofilm reactors for wastewater treatment, a comprehensive understanding of biofilm formation, reactor design, and the specific roles of microbes in the process is required. Worldwide, modifying biofilm reactors with different microbes has led to the significant removal of persistent pollutants, pharmaceuticals, and industrial wastewater components. However, before the large-scale deployment of biofilm reactors in Kathmandu Valley, an extensive evaluation of their performance in laboratory-scale settings over extended durations is crucial. Data obtained from these lab-scale systems can pinpoint potential operational challenges and inform optimization strategies, facilitating the seamless integration of such reactors into full-scale wastewater treatment facilities. Furthermore, investigations into the efficacy of engineered biofilm reactors harboring distinct microbial communities for wastewater treatment can be conducted. This targeted approach allows for the maximization of pollutant removal efficiency of specific wastewater composition, ultimately leading to the identification of their most suitable application sectors.

Machine learning approaches can be further explored to improve the efficiency of biofilm reactors and develop robust kinetic models for pollutant removal. The dynamic nature of municipal wastewater composition, characterized by significant temporal and seasonal fluctuations, presents a challenge. These variations can impact the influent load of organic and nutrient pollutants, introduce toxic compounds, and potentially lead to unforeseen metabolic pathways for pollutant removal. Consequently, these unconventional pathways necessitate detailed investigation at both the macroscopic (reactor level) and cellular levels. The biofilm's microbial community exhibits temporal dynamics due to the composition of influent wastewater, varying environmental conditions, and changes in operational parameters. Therefore, continuous monitoring of the reactor's microbial genome is crucial using different molecular biology techniques. A new direction for the study of biofilm technology may be developing strategies to sustain the population of specific beneficial microbes within the reactor.

Ethical Approval:

Not Applicable

Consent to Participate:

Not Applicable

Consent to Publish:

Not Applicable

Competing Interests:

The authors declare that they have no any competing interests.

Authors' Contribution

Writing original draft, Literature review, and Data Collection: **Pratap Bikram Shahi**; Supervision, Literature-Review and Editing: **Sarita Manandhar**; Conceptualization, Supervision, Writing-Review and Editing: **Shukra Raj Paudel**

Funding:

Funded by Aastha Scientific Research Service Pvt. Ltd.

Availability of data and materials:

All the data are provided in the manuscript.

References

1. Akhtar N, Syakir Ishak MI, Bhawani SA, Umar K. Various natural and anthropogenic factors responsible for water quality degradation: A review. Water. 2021;13(19):2660.
2. Khan R, Indhulekha K, Mawale Y, Dewangan R, Shekhar S, Dwivedi C, et al. Impact of anthropogenic activities on groundwater quality and quantity in Raipur City, Chhattisgarh, India. In IOP Publishing; 2020. p. 012006.
3. Khatri N, Tyagi S. Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. Frontiers in life science. 2015;8(1):23–39.
4. United Nations. Report of the Secretary-General, Progress towards the Sustainable Development Goals, E/2016/75. United Nations, New York, USA; 2016.
5. Haseena M, Malik M, Javed A, Arshad S, Asif N, Zulfiqar S, et al. Environmental Risk Assessment and Remediation. J Water pollution and human health. 2017;1(3):16–9.
6. WHO. Meeting the MDG drinking water and sanitation target: the urban and rural challenge of the decade. World Health Organization; 2006.
7. Thomas S, Gambrill M, Gilsdorf R, Diagne N. hard truths about the global sanitation crisis [WWW Document]. World Bank Blogs URL https://blogs worldbank org/water/3-hardtruths-about-global-sanitation-crisis (accessed 1111 19). 2018;
8. UN Water. Water and climate change. The United Nations World Water Development Report. 2020;
9. Wear SL, Acuña V, McDonald R, Font C. Sewage pollution, declining ecosystem health, and cross-sector collaboration. Biological Conservation. 2021;255:109010.
10. Hall N, Richards R, Barrington D, Ross H, Reid S, Head B, et al. Achieving the UN Sustainable Development Goals for water and beyond. 2016;
11. Jones ER, Van Vliet MTH, Qadir M, Bierkens MFP. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. Earth Syst Sci Data. 2021 Feb 8;13(2):237–54.
12. CBS. National Population and Housing Cencus 2021 [Internet]. National Planning Commission of Nepal. Government of Nepal, Kathmandu, Nepal.; 2021. Available from: https://censusnepal.cbs.gov.np/results/files/result-folder/Final_Population_compostion_12_2.pdf
13. Joshi DR. Urbanization Trend in Nepal. Contemporary Research: An Interdisciplinary Academic Journal. 2023;6(1):51–62.
14. Sapkota BD. Urbanization in Nepal: Focus on pre-requisites and trend. Journal of Population and Development. 2022;3(1):11–20.
15. Pandey CL. Managing urban water security: challenges and prospects in Nepal. Environment, Development and Sustainability. 2021;23(1):241–57.
16. Sarker B, Keya KN, Mahir FI, Nahiun KM, Shahida S, Khan RA. Surface and ground water pollution: causes and effects of urbanization and industrialization in South Asia. Scientific Review. 2021;7(3):32–41.
17. Devkota P, Dhakal S, Shrestha S, Shrestha UB. Land use land cover changes in the major cities of Nepal from 1990 to 2020. Environmental and Sustainability Indicators. 2023;17:100227.
18. JICA. Federal Democratic Republic of Nepal Data Collection Survey on Water Supply and Waste Water Sector in Nepal [Internet]. 2019. Available from: https://openjicareport.jica.go.jp/pdf/12326708_01.pdf
19. Karki BK, Baniya S, Kharel HL, Angove MJ, Paudel SR. Urban wastewater management in Nepal: generation, treatment, engineering and policy perspectives. H2Open Journal. 2024 Mar 1;7(2):222–42.
20. Koju NK, Sherpa CD, Koju NP. Assessment of physico-chemical parameters along with the concentration of heavy metals in the effluents released from different industries in Kathmandu valley. Water, Air, & Soil Pollution. 2022;233(5):176.
21. Bilal M, Shah JA, Ashfaq T, Gardazi SMH, Tahir AA, Pervez A, et al. Waste biomass adsorbents for copper removal from industrial wastewater—a review. Journal of hazardous materials. 2013;263:322–33.
22. Mojiri A, Bashir MJK. Wastewater Treatment: Current and Future Techniques. Water. 2022 Feb 1;14(3):448.
23. Abidin ZZ, Shamsudin NSM, Madehi N, Sobri S. Optimisation of a method to extract the active coagulant agent from *Jatropha curcas* seeds for use in turbidity removal. Industrial Crops and Products. 2013;41:319–23.
24. Kakoi B, Kaluli JW, Ndiba P, Thiong'o G. Banana pith as a natural coagulant for polluted river water. Ecological engineering. 2016;95:699–705.
25. Crini G, Lichtfouse E. Advantages and disadvantages of techniques used for wastewater treatment. Environmental chemistry letters. 2019;17:145–55.
26. Bahrodin MB, Zaidi NS, Hussein N, Sillanpää M, Prasetyo DD, Syafiuddin A. Recent advances on coagulation-based treatment of wastewater: Transition from chemical to natural coagulant. Current Pollution Reports. 2021;7(3):379–91.
27. Boulaadjoul S, Zemmouri H, Bendjama Z, Drouiche N. A novel use of Moringa oleifera seed powder in enhancing the primary treatment of paper mill effluent. Chemosphere. 2018;206:142–9.
28. Alsvik IL, Hägg MB. Pressure retarded osmosis and forward osmosis membranes: materials and methods. Polymers. 2013;5(1):303–27.
29. Goh PS, Wong TW, Lim JW, Ismail AF, Hilal N. Innovative and sustainable membrane technology for wastewater treatment and desalination application. In: Innovation strategies in environmental science. Elsevier; 2020. p. 291–319.
30. Gebru KA, Das C. Removal of chromium (VI) ions from aqueous solutions using amine-impregnated TiO_2 nanoparticles modified cellulose acetate membranes. Chemosphere. 2018;191:673–84.
31. Bera SP, Godhaniya M, Kothari C. Emerging and advanced membrane technology for wastewater treatment: A review. Journal of Basic Microbiology. 2022;62(3–4):245–59.
32. Sadeghfar F, Ghaedi M, Zalipour Z. Chapter 4 - Advanced oxidation. In: Ghaedi M, editor. Interface Science and Technology [Internet]. Elsevier; 2021. p. 225–324. Available from: https://www.sciencedirect.com/science/article/pii/B9780128188064000012
33. Agulló-Barceló M, Polo-López MI, Lucena F, Jofre J, Fernández-Ibánez P. Solar advanced oxidation processes as disinfection tertiary treatments for real wastewater: implications for water reclamation. Applied Catalysis B: Environmental. 2013;136:341–50.
34. Brienza M, Katsoyiannis IA. Sulfate radical technologies as tertiary treatment for the removal of emerging contaminants from wastewater. Sustainability. 2017;9(9):1604.
35. Doltade SB, Yadav YJ, Jadhav NL. Industrial wastewater treatment using oxidative integrated approach. South African Journal of Chemical Engineering. 2022;40(1):100–6.
36. Tchobanoglous G, Burton FL, Stensel HD, Metcalf & Eddy Inc. Wastewater Engineering: Treatment and Reuse [Internet]. McGraw-Hill Education; 2003. (McGraw-Hill higher education). Available from: https://books.google.com.np/books?id=-eoeAQAAIAAJ
37. Aqeel H, Weissbrodt DG, Cerruti M, Wolfaardt GM, Wilén BM, Liss SN. Drivers of bioaggregation from flocs to biofilms and granular

sludge. Environmental Science: Water Research & Technology. 2019;5(12):2072–89.
38. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010 Sep;8(9):623–33.
39. Hedaoo MN, Bhole AG, Ingole NW, Hung YT. Biological wastewater treatment. In: Handbook of environment and waste management: Air and water pollution control. World Scientific; 2012. p. 431–73.
40. Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F, Nava-Saucedo JE. Polymer biodegradation: Mechanisms and estimation techniques–A review. Chemosphere. 2008;73(4):429–42.
41. Willey J, Sandman K, Wood D. Prescott's microbiology. 11th ed. McGraw-Hill; 2019.
42. Speight JG, Arjoon KK. Bioremediation of petroleum and petroleum products. John Wiley & Sons; 2012.
43. Bhatt P, Pathak VM, Joshi S, Bisht TS, Singh K, Chandra D. Major metabolites after degradation of xenobiotics and enzymes involved in these pathways. In: Smart bioremediation technologies. 2019. p. 205–15.
44. Eskander S, Saleh H. Biodegradation: process mechanism. Environ Sci & Eng. 2017;8(8):1–31.
45. Moodie AD, Ingledew WJ. Microbial anaerobic respiration. Advances in microbial physiology. 1990;31:225–69.
46. Reineke W. Aerobic and anaerobic biodegradation potentials of microorganisms. Biodegradation and Persistence. 2001;1–161.
47. Machineni L. Review on biological wastewater treatment and resources recovery: attached and suspended growth systems. Water science and technology. 2019;80(11):2013–26.
48. Saravanan A, Kumar PS, Varjani S, Jeevanantham S, Yaashikaa PR, Thamarai P, et al. A review on algal-bacterial symbiotic system for effective treatment of wastewater. Chemosphere. 2021 May;271:129540.
49. Sehar S, Naz I. Role of the Biofilms in Wastewater Treatment. In: Dhanasekaran D, Thajuddin N, editors. Microbial Biofilms - Importance and Applications [Internet]. InTech; 2016 [cited 2024 Jan 23]. Available from: http://www.intechopen.com/books/microbial-biofilms-importance-and-applications/role-of-the-biofilms-in-wastewater-treatment
50. Kokare C, Chakraborty S, Khopade A, Mahadik KR. Biofilm: Importance and applications. 2009;
51. Chattopadhyay I, Usman TM, Varjani S. Exploring the role of microbial biofilm for industrial effluents treatment. Bioengineered. 2022;13(3):6420–40.
52. Saini S, Tewari S, Dwivedi J, Sharma V. Biofilm-mediated wastewater treatment: a comprehensive review. Mater Adv. 2023;4(6):1415–43.
53. Barkay T, Schaefer J. Metal and radionuclide bioremediation: issues, considerations and potentials. Current opinion in microbiology. 2001;4(3):318–23.
54. Lewandowski Z, Boltz JP. Biofilms in water and wastewater treatment. In 2011.
55. Gurung SB, Geronimo FKF, Lee S, Kim LH. Status of constructed wetlands in Nepal: recent developments and future concerns. Journal of Wetlands Research. 2017;19(1):45–51.
56. Bista K, Khatiwada N. Performance study on reed bed wastewater treatment units in Nepal. In 2004. p. 12–5.
57. Lesiv M, Polishchuk A, Antonyak H. Aquatic macrophytes: ecological features and functions. Studia Biologica. 2020;14(2):79–94.
58. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annual review of microbiology. 1995;49(1):711–45.
59. Danhorn T, Fuqua C. Biofilm formation by plant-associated bacteria. Annu Rev Microbiol. 2007;61:401–22.
60. Otte ML, Jacob DL. Constructed wetlands for phytoremediation: rhizofiltration, phytostabilisation and phytoextraction. In: Phytoremediation rhizoremediation. Springer; 2006. p. 57–67.
61. Boukalová Z, Těšitel J, Gurung BD. Constructed wetlands implementation in Kathmandu Valley, Nepal. International Journal of Environmental Impacts. 2021;4(4):363–74.
62. Ghimire A, Kumar K, Thapa B. Design Approach for Sub-surface Flow Constructed Wetlands. Hydro Nepal: Journal of Water, Energy & Environment. 2012;(10).
63. Ojha B. Constructed Wetland as a Leachate Treatment Option in Nepal. SCITECH Nepal. 2020;15(1):49–53.
64. Horan N. Suspended growth processes. The Handbook of Water and Wastewater Microbiology. 2003;351–60.
65. Ghangrekar M, Behera M. Suspended growth treatment processes. 2014;
66. Davis ML. Secondary treatment by suspended growth biological processes. In: Water and Wastewater Engineering: Design Principles and Practice. First edition. New York: McGraw-Hill; 2010. p. 23–3.
67. Spellman FR. Handbook of environmental engineering. Crc Press; 2023.
68. Yildiz BS. Water and wastewater treatment: Biological processes. In: Metropolitan Sustainability. Elsevier; 2012. p. 406–28.
69. Wang LK, Wu Z, Shammas NK. Activated Sludge Processes [Internet]. Wang LK, Pereira NC, Hung YT, editors. Biological Treatment Processes. Totowa, NJ: Humana Press; 2009. p. 207–81. Available from: https://doi.org/10.1007/978-1-60327-156-1_6
70. Chai Q, Bakke R, Lie B. Object-oriented modeling and optimal control of a biological wastewater treatment process. In 2006. p. 218–23.
71. Amanatidou E, Samiotis G, Trikoilidou E, Tzelios D, Michailidis A. Influence of wastewater treatment plants' operational conditions on activated sludge microbiological and morphological characteristics. Environmental technology. 2016;37(2):265–78.
72. Ye F, Ye Y, Li Y. Effect of C/N ratio on extracellular polymeric substances (EPS) and physicochemical properties of activated sludge flocs. Journal of hazardous materials. 2011;188(1–3):37–43.
73. Abou-Elela SI, Kamel MM, Fawzy ME. Biological treatment of saline wastewater using a salt-tolerant microorganism. Desalination. 2010;250(1):1–5.
74. Sparchez E, Elefsiniotis P, Wareham D, Fongsatitkul P. Co-treatment of domestic and dairy wastewater in an activated sludge system. Environmental technology. 2015;36(6):715–21.
75. Ding S, Bao P, Wang B, Zhang Q, Peng Y. Long-term stable simultaneous partial nitrification, anammox and denitrification (SNAD) process treating real domestic sewage using suspended activated sludge. Chemical Engineering Journal. 2018;339:180–8.
76. Ng J, Wong D, Kutty S, Jagaba A. Organic and nutrient removal for domestic wastewater treatment using bench-scale sequencing batch reactor. In AIP Publishing; 2021.
77. Jagaba AH, Kutty SRM, Baloo L, Noor A, Abubakar S, Lawal IM, et al. Effect of hydraulic retention time on the treatment of pulp and paper industry wastewater by extended aeration activated sludge system. In IEEE; 2021. p. 221–4.
78. Norvill ZN, Toledo-Cervantes A, Blanco S, Shilton A, Guieysse B, Muñoz R. Photodegradation and sorption govern tetracycline removal during wastewater treatment in algal ponds. Bioresource technology. 2017;232:35–43.
79. Dinh TTU, Soda S, Nguyen TAH, Nakajima J, Cao TH. Nutrient removal by duckweed from anaerobically treated swine wastewater in lab-scale stabilization ponds in Vietnam. Science of the total environment. 2020;722:137854.
80. Remy M, Potier V, Temmink H, Rulkens W. Why low powdered activated carbon addition reduces membrane fouling in MBRs. Water Research. 2010;44(3):861–7.
81. Ji J, Chen Y, Hu Y, Ohtsu A, Ni J, Li Y, et al. One-year operation of a 20-L submerged anaerobic membrane bioreactor for real domestic wastewater treatment at room temperature: Pursuing the optimal HRT and sustainable flux. Science of the Total Environment. 2021;775:145799.
82. Isik O, Batyrow M, Abdelrahman AM, Orman I, Ozgun H, Ersahin ME, et al. Dynamic membrane bioreactor performance for treatment of municipal wastewaters at different sludge concentrations. Environmental Technology & Innovation. 2021;22:101452.
83. Copelli S, Raboni M, Urbini G. Water pollution: biological oxidation and natural control techniques. In: Reference module in chemistry, molecular sciences and chemical engineering. Elsevier; 2015. p. 1–28.
84. Rani R, Singh S. Green chemistry and its applications in hospital wastewater and its treatment. In: Green Chemistry and Water Remediation: Research and Applications. Elsevier; 2021. p. 271–98.
85. Mara D. Waste stabilization ponds: Past, present and future. Desalination and Water Treatment. 2009;4(1–3):85–8.
86. Spellman FR, Drinan J. Wastewater stabilization ponds. Vol. 657. CRC Press Boca Raton; 2014.
87. Kadri SUT, Tavanappanavar AN, Nagesh Babu R, Bilal M, Singh B, Gupta SK, et al. Overview of Waste Stabilization Ponds in Developing Countries. In: Cost-efficient Wastewater Treatment Technologies: Natural Systems. Springer; 2021. p. 153–75.
88. Ranjit P, Jhansi V, Reddy KV. Conventional wastewater treatment processes. Advances in the Domain of Environmental Biotechnology: Microbiological Developments in Industries, Wastewater Treatment and Agriculture. 2021;455–79.
89. Mara D. Domestic wastewater treatment in developing countries. Routledge; 2013.

90. Andreoli CV, Von Sperling M, Fernandes F. Sludge treatment and disposal. IWA publishing; 2007.
91. Mara DD, Pearson HW. Waste stabilization ponds: design manual for Mediterranean Europe. Vol. 20. World Health Organization, Regional Office for Europe; 1987.
92. Wen G, Ma J, Zhang L, Yu G. 4.07 - Membrane Bioreactor in Water Treatment. In: Drioli E, Giorno L, editors. Comprehensive Membrane Science and Engineering [Internet]. Oxford: Elsevier; 2010. p. 195–209. Available from: https://www.sciencedirect.com/science/article/pii/B9780080932507000323
93. Al-Asheh S, Bagheri M, Aidan A. Membrane bioreactor for wastewater treatment: A review. Case Studies in Chemical and Environmental Engineering. 2021;4:100109.
94. Ng AN, Kim AS. A mini-review of modeling studies on membrane bioreactor (MBR) treatment for municipal wastewaters. Desalination. 2007;212(1–3):261–81.
95. Judd S. The status of membrane bioreactor technology. Trends in biotechnology. 2008;26(2):109–16.
96. Koul B, Yadav D, Singh S, Kumar M, Song M. Insights into the domestic wastewater treatment (DWWT) regimes: a review. Water. 2022;14(21):3542.
97. Mesquita D, Amaral A, Ferreira EC. Characterization of activated sludge abnormalities by image analysis and chemometric techniques. Analytica chimica acta. 2011;705(1–2):235–42.
98. Mesquita DP, Amaral AL, Ferreira EC. Activated sludge characterization through microscopy: A review on quantitative image analysis and chemometric techniques. Analytica Chimica Acta. 2013;802:14–28.
99. Oller I, Gernjak W, Maldonado M, Fernandez-Ibanez P, Blanco J, Sanchez-Perez J, et al. Degradation of the insecticide dimethoate by solar photocatalysis at pilot plant scale. Environ Chem Lett. 2005;3:118–21.
100. dos Santos SL, van Haandel A. Transformation of waste stabilization ponds: Reengineering of an obsolete sewage treatment system. Water. 2021;13(9):1193.
101. Ghodeif K. Baseline Assessment Study for Wastewater Treatment Plant for Al Gozayyera Village, West Kantara City, Ismailia Governorate, Egypt. Network of demonstration activities for sustainable integrated wastewater treatment and reuse in the Mediterranean: Cairo, Egypt. 2013;
102. Rasheed A, Ciroma S. A Review on Sequencing Batch Reactors: Process Design, Operation and Modelling. 2020;
103. Roccaro P, Vagliasindi FG. Membrane bioreactors for wastewater reclamation: Cost analysis. In: Current Developments in Biotechnology and Bioengineering. Elsevier; 2020. p. 311–22.
104. Shrestha P, Shrestha R, Dangol B. Status of wastewater generation and management in urban Nepal. Journal of Environment and Public Health. 2017;1:1–6.
105. KUKL. Annual Report 2080 [Internet]. Kathmandu Upatyaka Khanepani Limited; 2023. Available from: https://kathmanduwater.org/
106. Sharafi K, Fazlzadeh Davil M, Heidari M, Almasi A, Taheri H. Comparison of conventional activated sludge system and stabilization pond in removal of chemical and biological parameters. International Journal of Environmental Health Engineering. 2012;1(October):1–5.
107. US EPA. Wastewater Technology Fact Sheet [Internet]. 2000 [cited 2024 Apr 28]. Available from: https://www.epa.gov/septic/wastewater-technology-fact-sheets
108. Barr TA, Taylor JM, Duff SJ. Effect of HRT, SRT and temperature on the performance of activated sludge reactors treating bleached kraft mill effluent. Water research. 1996;30(4):799–810.
109. Ghimire S, Pokhrel N, Pant S, Gyawali T, Koirala A, Mainali B, et al. Assessment of technologies for water quality control of the Bagmati River in Kathmandu valley, Nepal. Groundwater for Sustainable Development. 2022 Aug;18:100770.
110. Zhao L, Dai T, Qiao Z, Sun P, Hao J, Yang Y. Application of artificial intelligence to wastewater treatment: A bibliometric analysis and systematic review of technology, economy, management, and wastewater reuse. Process Safety and Environmental Protection. 2020;133:169–82.
111. Boltz JP, Smets BF, Rittmann BE, Van Loosdrecht MC, Morgenroth E, Daigger GT. From biofilm ecology to reactors: a focused review. Water Science and Technology. 2017;75(8):1753–60.
112. George R, Muraleedharan P, Parvathavarthini N, Khatak H, Rao T. Microbiologically influenced corrosion of AISI type 304 stainless steels under fresh water biofilms. Materials and Corrosion. 2000;51(4):213–8.
113. Yin W, Wang Y, Liu L, He J. Biofilms: the microbial “protective clothing” in extreme environments. International journal of molecular sciences. 2019;20(14):3423.
114. Characklis WG, Cooksey KE. Biofilms and Microbial Fouling. In: Laskin AI, editor. Advances in Applied Microbiology [Internet]. Academic Press; 1983. p. 93–138. Available from: https://www.sciencedirect.com/science/article/pii/S0065216408703551
115. Morgenroth E. Modelling biofilms. In: Chen G, van Loosdrecht MCM, Ekama GA, Brdjanovic D, editors. Biological Wastewater Treatment: Principles, Modelling and Design [Internet]. IWA Publishing; 2023 [cited 2024 Jan 23]. p. 0. Available from: https://doi.org/10.2166/9781789060362_0757
116. Stewart PS. Diffusion in Biofilms. J Bacteriol. 2003 Mar;185(5):1485–91.
117. Sørensen KH, Morgenroth E. Biofilm reactors. Biological wastewater treatment: principles, modelling and design, 2nd edn IWA Publishing, London. 2020;813–39.
118. Espinosa-Ortiz EJ, Gerlach R, Peyton BM, Roberson L, Yeh DH. Biofilm reactors for the treatment of used water in space: potential, challenges, and future perspectives. Biofilm. 2023;6:100140.
119. Mitra A, Mukhopadhyay S. Biofilm mediated decontamination of pollutants from the environment. Aims Bioengineering. 2016;3(1):44–59.
120. Naz I, Saroj DP, Mumtaz S, Ali N, Ahmed S. Assessment of biological trickling filter systems with various packing materials for improved wastewater treatment. Environmental Technology. 2015;36(4):424–34.
121. WEF. Design of Water Resource Recovery Facilities, MOP 8. WEF Press (Water Environment Federation and McGraw Hill Education); 2018.
122. Gray NF. Fixed-film reactors in wastewater treatment. World Scientific; 2020.
123. Murshid S, Antonysamy A, Dhakshinamoorthy G, Jayaseelan A, Pugazhendhi A. A review on biofilm-based reactors for wastewater treatment: Recent advancements in biofilm carriers, kinetics, reactors, economics, and future perspectives. Science of The Total Environment. 2023 Sep;892:164796.
124. Chen GH, van Loosdrecht MC, Ekama GA, Brdjanovic D. Biological wastewater treatment: principles, modeling and design. IWA publishing; 2020.
125. Santos AD, Martins RC, Quinta-Ferreira RM, Castro LM. Moving bed biofilm reactor (MBBR) for dairy wastewater treatment. Energy Reports. 2020;6:340–4.
126. Ødegaard H. Innovations in wastewater treatment:–the moving bed biofilm process. Water science and technology. 2006;53(9):17–33.
127. di Biase A, Kowalski MS, Devlin TR, Oleszkiewicz JA. Moving bed biofilm reactor technology in municipal wastewater treatment: a review. Journal of environmental management. 2019;247:849–66.
128. Delatolla R, Tufenkji N, Comeau Y, Gadbois A, Lamarre D, Berk D. Effects of long exposure to low temperatures on nitrifying biofilm and biomass in wastewater treatment. Water Environment Research. 2012;84(4):328–38.
129. Cui YX, Wu D, Mackey HR, Chui HK, Chen GH. Application of a moving-bed biofilm reactor for sulfur-oxidizing autotrophic denitrification. Water Science and Technology. 2018;77(4):1027–34.
130. Kanwar RMA, Khan ZM, Farid HU. Investigation of municipal wastewater treatment by agricultural waste materials in locally designed trickling filter for peri-urban agriculture. Water Supply. 2021;21(5):2298–312.
131. Paixão Filho JL da, Portela DG, da Silva CI, de Oliveira GC, Tonetti AL. Use of expanded polystyrene (EPS) as a support media for trickling filters applied to wastewater treatment. Applied Water Science. 2023;13(2):66.
132. El-Shafai SA, Zahid WM. Performance of aerated submerged biofilm reactor packed with local scoria for carbon and nitrogen removal from municipal wastewater. Bioresource technology. 2013;143:476–82.
133. Carneiro RB, Gomes GM, Zaiat M, Santos-Neto ÁJ. Two-phase (acidogenic-methanogenic) anaerobic fixed bed biofilm reactor enhances the biological domestic sewage treatment: Perspectives for recovering bioenergy and value-added by-products. Journal of Environmental Management. 2022 Sep 1;317:115388.
134. Wang F, Zhou L, Zhao J. The performance of biocarrier containing zinc nanoparticles in biofilm reactor for treating textile wastewater. Process Biochemistry. 2018 Nov 1;74:125–31.
135. Gustavsson DJI, Suarez C, Wilén BM, Hermansson M, Persson F. Long-term stability of partial nitritation-anammox for treatment of municipal wastewater in a moving bed biofilm reactor pilot system. Science of The Total Environment. 2020 Apr 20;714:136342.

136. Boelee NC, Temmink H, Janssen M, Buisman CJ, Wijffels RH. Scenario analysis of nutrient removal from municipal wastewater by microalgal biofilms. Water. 2012;4(2):460–73.
137. Chen S, Xie J, Wen Z. Removal of pharmaceutical and personal care products (PPCPs) from waterbody using a revolving algal biofilm (RAB) reactor. Journal of Hazardous Materials. 2021;406:124284.
138. Chen CL, Huang CC, Ho KC, Hsiao PX, Wu MS, Chang JS. Biodiesel production from wet microalgae feedstock using sequential wet extraction/transesterification and direct transesterification processes. Bioresource technology. 2015;194:179–86.
139. Chiellini E, Cinelli P, Ilieva VI, Martera M. Biodegradable thermoplastic composites based on polyvinyl alcohol and algae. Biomacromolecules. 2008;9(3):1007–13.
140. Christenson LB, Sims RC. Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products. Biotechnology and bioengineering. 2012;109(7):1674–84.
141. Posadas E, García-Encina PA, Soltau A, Domínguez A, Díaz I, Muñoz R. Carbon and nutrient removal from centrates and domestic wastewater using algal–bacterial biofilm bioreactors. Bioresource technology. 2013;139:50–8.
142. He S, Xue G. Algal-based immobilization process to treat the effluent from a secondary wastewater treatment plant (WWTP). Journal of hazardous materials. 2010;178(1–3):895–9.
143. Boelee NC, Temmink H, Janssen M, Buisman C, Wijffels R. Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. Water research. 2011;45(18):5925–33.
144. Gao L, Han F, Zhang X, Liu B, Fan D, Sun X, et al. Simultaneous nitrate and dissolved organic matter removal from wastewater treatment plant effluent in a solid-phase denitrification biofilm reactor. Bioresource Technology. 2020;314:123714.
145. Zhang D, Liu Y, Han Y, Zhang Y, Jia X, Li W, et al. Nitrate removal from low C/N wastewater at low temperature by immobilized *Pseudomonas* sp. Y39-6 with versatile nitrate metabolism pathways. Bioresource Technology. 2021;326:124794.
146. Khosravi A, Javdan M, Yazdanpanah G, Malakootian M. Removal of heavy metals by *Escherichia coli* (*E. coli*) biofilm placed on zeolite from aqueous solutions (case study: the wastewater of Kerman Bahonar Copper Complex). Appl Water Sci. 2020 Jul;10(7):167.
147. Begum SS, Radha K. Gas–liquid mass transfer studies in inverse fluidized bed biofilm reactor for the biodegradation of industrial effluent rich in phenolic compounds. Environmental Progress & Sustainable Energy. 2016;35(2):433–8.
148. Yadav KK, Mandal AK, Sen IK, Chakraborti S, Islam SS, Chakraborty R. Flocculating property of extracellular polymeric substances produced by a biofilm-forming bacterium *Acinetobacter junii* BB1A. Applied biochemistry and biotechnology. 2012;168:1621–34.
149. Tang CC, Tian Y, Liang H, Zuo W, Wang ZW, Zhang J, et al. Enhanced nitrogen and phosphorus removal from domestic wastewater via algae-assisted sequencing batch biofilm reactor. Bioresource Technology. 2018 Feb;250:185–90.
150. Amini E, Babaei A, Mehrnia MR, Shayegan J, Safdari MS. Municipal wastewater treatment by semi-continuous and membrane algal-bacterial photo-bioreactors. Journal of Water Process Engineering. 2020;36:101274.
151. Katam K, Shimizu T, Soda S, Bhattacharyya D. Performance evaluation of two trickling filters removing LAS and caffeine from wastewater: Light reactor (algal-bacterial consortium) vs dark reactor (bacterial consortium). Science of The Total Environment. 2020 Mar 10;707:135987.
152. De Godos I, González C, Becares E, García-Encina PA, Muñoz R. Simultaneous nutrients and carbon removal during pretreated swine slurry degradation in a tubular biofilm photobioreactor. Applied microbiology and biotechnology. 2009;82:187–94.
153. Esfahani EB, Zeidabadi FA, Bazargan A, McKay G. The modified Bardenpho process. Handbook of environmental materials management. 2018;1–50.
154. Escudié R, Cresson R, Delgenès JP, Bernet N. Control of start-up and operation of anaerobic biofilm reactors: an overview of 15 years of research. Water research. 2011;45(1):1–10.
155. Asri M, Elabed S, Ibnsouda Koraichi S, El Ghachtouli N. Biofilm-Based Systems for Industrial Wastewater Treatment. In: Hussain CM, editor. Handbook of Environmental Materials Management [Internet]. Cham: Springer International Publishing; 2019 [cited 2024 Jan 23]. p. 1767–87. Available from: http://link.springer.com/10.1007/978-3-319-73645-7_137
156. Kesaano M, Sims RC. Algal biofilm based technology for wastewater treatment. Algal Research. 2014 Jul;5:231–40.
157. Characklis WG. Physiological ecology in biofilm systems. Biofilms. 1990;
158. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical microbiology reviews. 2002;15(2):167–93.
159. Qureshi N, Maddox I. Novel bioreactors for the ABE fermentation using cells of Clostridium acetobutylicum immobilized by adsorption onto bonechar. Fermentation technologies: Industrial applications Edited by: Yu PL London: Elsevier Appl Sci Publ. 1990;
160. Lu A, Gao Y, Jin T, Luo X, Zeng Q, Shang Z. Effects of surface roughness and texture on the bacterial adhesion on the bearing surface of bio-ceramic joint implants: An in vitro study. Ceramics International. 2020 Apr 1;46(5):6550–9.
161. Hsieh PC, Chien HW. Biomimetic surfaces: Insights on the role of surface topography and wetting properties in bacterial attachment and biofilm formation. Colloids and Surfaces B: Biointerfaces. 2023;228:113389.
162. Zhang Q, Yu Z, Jin S, Liu C, Li Y, Guo D, et al. Role of surface roughness in the algal short-term cell adhesion and long-term biofilm cultivation under dynamic flow condition. Algal Research. 2020 Mar 1;46:101787.
163. Danaee S, Ofoghi H, Heydarian SM. Acceleration of microalgal biofilm formation on PET by surface engineering. Korean Journal of Chemical Engineering. 2021;38:2500–9.
164. Annachhatre A, Bhamidimarri S. Microbial attachment and growth in fixed-film reactors: process startup considerations. Biotechnology advances. 1992;10(1):69–91.
165. Qureshi N, Annous BA, Ezeji TC, Karcher P, Maddox IS. Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. Microbial cell factories. 2005;4:1–21.
166. Adetunji VO, Odetokun IA. Biofilm formation in human and tropical foodborne isolates of Listeria strains. American Journal of Food Technology. 2012;7:517–31.
167. Garrett TR, Bhakoo M, Zhang Z. Bacterial adhesion and biofilms on surfaces. Progress in natural science. 2008;18(9):1049–56.
168. Ponomareva A, Buzoleva L, Bogatyrenko E. Abiotic environmental factors affecting the formation of microbial biofilms. Biology Bulletin. 2018;45:490–6.
169. Morimatsu K, Eguchi K, Hamanaka D, Tanaka F, Uchino T. Effects of temperature and nutrient conditions on biofilm formation of *Pseudomonas putida*. Food Science and Technology Research. 2012;18(6):879–83.
170. Li W, Siddique MS, Graham N, Yu W. Influence of temperature on biofilm formation mechanisms using a gravity-driven membrane (GDM) system: insights from microbial community structures and metabolomics. Environmental Science & Technology. 2022;56(12):8908–19.
171. Ras M, Steyer JP, Bernard O. Temperature effect on microalgae: a crucial factor for outdoor production. Reviews in environmental science and bio/technology. 2013;12(2):153–64.
172. Gonzalez-Camejo J, Aparicio S, Ruano M, Borrás L, Barat R, Ferrer J. Effect of ambient temperature variations on an indigenous microalgae-nitrifying bacteria culture dominated by *Chlorella*. Bioresource technology. 2019;290:121788.
173. DWSSM. Department of Water Supply and Sewerage Management [Internet]. 2023 [cited 2024 Jan 23]. Available from: https://dwssm.gov.np/
174. Oliveira R, Melo L, Oliveira A, Salgueiro R. Polysaccharide production and biofilm formation by *Pseudomonas fluorescens*: effects of pH and surface material. 1994;
175. Bogino PC, de las Mercedes Oliva M, Sorroche FG, Giordano W. The role of bacterial biofilms and surface components in plant-bacterial associations. International journal of molecular sciences. 2013;14(8):15838–59.
176. Maleki A, Hayati B, Najafi F, Gharibi F, Joo SW. Heavy metal adsorption from industrial wastewater by PAMAM/TiO_2 nanohybrid: preparation, characterization and adsorption studies. Journal of Molecular Liquids. 2016;224:95–104.
177. Zmantar T, Kouidhi B, Miladi H, Mahdouani K, Bakhrouf A. A microtiter plate assay for *Staphylococcus aureus* biofilm quantification at various pH levels and hydrogen peroxide supplementation. The new microbiologica. 2010;33(2):137.
178. Li X, Lu Y, Luo H, Liu G, Torres CI, Zhang R. Effect of pH on bacterial distributions within cathodic biofilm of the microbial fuel cell with maltodextrin as the substrate. Chemosphere. 2021 Feb 1;265:129088.
179. Roy PK, Ha AJW, Mizan MFR, Hossain MI, Ashrafudoulla M, Toushik SH, et al. Effects of environmental conditions (temperature, pH, and

glucose) on biofilm formation of *Salmonella enterica* serotype Kentucky and virulence gene expression. Poultry Science. 2021;100(7):101209.

180. Zhang HY, Kuang YL, Lin Z, Liu CH. Influence on surface characteristics of microalgae cell by solution chemistry. Advanced Materials Research. 2011;287:1938–42.
181. Zhao Z, Muylaert K, Szymczyk A, Vankelecom IFJ. Enhanced microalgal biofilm formation and facilitated microalgae harvesting using a novel pH-responsive, crosslinked patterned and vibrating membrane. Chemical Engineering Journal. 2021 Apr 15;410:127390.
182. Simoes M, Pereira MO, Sillankorva S, Azeredo J, Vieira MJ. The effect of hydrodynamic conditions on the phenotype of *Pseudomonas fluorescens* biofilms. Biofouling. 2007;23(4):249–58.
183. Khu ST, Changchun X, Wang T. Effects of flow velocity on biofilm composition and microbial molecular ecological network in reclaimed water distribution systems. Chemosphere. 2023 Nov 1;341:140010.
184. Roosjen A, Boks NP, van der Mei HC, Busscher HJ, Norde W. Influence of shear on microbial adhesion to PEO-brushes and glass by convective-diffusion and sedimentation in a parallel plate flow chamber. Colloids and Surfaces B: Biointerfaces. 2005 Nov 25;46(1):1–6.
185. Siddiqui S, Chandrasekaran A, Lin N, Tufenkji N, Moraes C. Microfluidic shear assay to distinguish between bacterial adhesion and attachment strength on stiffness-tunable silicone substrates. Langmuir. 2019;35(26):8840–9.
186. Chang J, He X, Bai X, Yuan C. The impact of hydrodynamic shear force on adhesion morphology and biofilm conformation of *Bacillus* sp. Ocean Engineering. 2020 Feb 1;197:106860.
187. Navada S, Knutsen MF, Bakke I, Vadstein O. Nitrifying biofilms deprived of organic carbon show higher functional resilience to increases in carbon supply. Scientific reports. 2020;10(1):7121.
188. Mohiuddin SS, Khattar D. Biochemistry, ammonia. StatPearls Publishing, Treasure Island (FL) [Internet]. 2023; Available from: https://europepmc.org/article/nbk/nbk541039#impact
189. Rojo F. Carbon catabolite repression in *Pseudomonas*: optimizing metabolic versatility and interactions with the environment. FEMS microbiology reviews. 2010;34(5):658–84.
190. Alleman JE. Elevated nitrite occurrence in biological wastewater treatment systems. Water Science and Technology. 1985 Feb 1;17(2–3):409–19.
191. Yang Y, Niu Q, Lu J, Li Z, Yang B, Lei L, et al. The inhibitory effects and underlying mechanism of high ammonia stress on sulfide-driven denitrification process. Chemosphere. 2022;303:135093.
192. Al-Sayed A, Hassan GK, Al-Shemy MT, El-gohary FA. Effect of organic loading rates on the performance of membrane bioreactor for wastewater treatment behaviors, fouling, and economic cost. Sci Rep. 2023 Sep 20;13(1):15601.
193. Fallahi A, Rezvani F, Asgharnejad H, Nazloo EK, Hajinajaf N, Higgins B. Interactions of microalgae-bacteria consortia for nutrient removal from wastewater: A review. Chemosphere. 2021;272:129878.
194. Gonzalez-Camejo J, Barat R, Pachés M, Murgui M, Seco A, Ferrer J. Wastewater nutrient removal in a mixed microalgae–bacteria culture: effect of light and temperature on the microalgae–bacteria competition. Environmental Technology. 2018;39(4):503–15.
195. Jin Y, Ding J, Zhan W, Du J, Wang G, Pang J, et al. Effect of dissolved oxygen concentration on performance and mechanism of simultaneous nitrification and denitrification in integrated fixed-film activated sludge sequencing batch reactors. Bioresource Technology. 2023;387:129616.
196. Ting Y, Prince I, Lawson F. Uptake of cadmium and zinc by the alga Chlorella vulgaris: II. Multi-ion situation. Biotechnology and Bioengineering. 1991;37(5):445–55.