Review

Exploiting Biofilm Characteristics to Enhance Biological Nutrient Removal in Wastewater Treatment Plants

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Abstract: Biological treatments are integral processes in wastewater treatment plants (WWTPs). They can be carried out using sludge or biofilm processes. Although the sludge process is effective for biological wastewater systems, it has some drawbacks that make it undesirable. Hence, biofilm processes have gained popularity, since they address the drawbacks of sludge treatments, such as the high rates of sludge production. Although biofilms have been reported to be essential for wastewater, few studies have reviewed the different ways in which the biofilm properties can be explored, especially for the benefit of wastewater treatment. Thus, this review explores the properties of biofilms that can be exploited to enhance biological wastewater systems. In this review, it is revealed that various biofilm properties, such as the extracellular polymeric substances (EPS), quorum sensing (Qs), and acylated homoserine lactones (AHLs), can be enhanced as a sustainable and cost-effective strategy to enhance the biofilm. Moreover, the exploitation of other biofilm properties such as the SOS, which is only reported in the medical field, with no literature reporting it in the context of wastewater treatment, is also recommended to improve the biofilm technology for wastewater treatment processes. Additionally, this review further elaborates on ways that these properties can be exploited to advance biofilm wastewater treatment systems. A special emphasis is placed on exploiting these properties in simultaneous nitrification and denitrification and biological phosphorus removal processes, which have been reported to be the most sensitive processes in biological wastewater treatment.

Keywords: biofilm; bacteria; nitrification; denitrification; exopolymeric substances

1. Introduction

The biological treatment is one of the fundamental processes for the treatment of contaminated water in WWTPs, as it mainly involves the removal of toxic contaminants such as inorganic nitrogen and phosphorus. It is a preferred method for the removal of inorganic impurities in various municipal and industrial WWTPs. Biological treatment is ideal for inorganic contaminants due to the benefit it offers over physical and chemical methods. Among the many benefits of biological treatment, its economic advantages and environmental friendliness are the main attractive benefits of this process. The use of activated sludge has been a popular biological treatment process for centuries in WWTPs [1,2]. Although the sludge process is an efficient process for the treatment of dissolved contaminants, including total nitrogen and phosphorus compounds, it has its drawbacks, such as the production of excess sludge. The post-treatment and disposal of the sludge are other challenges when using sludge processes. Declines in available land space, coupled with increasingly stringent regulations governing the design and operation of new landfills, have caused the costs of siting, building, and operating new landfills to rise sharply, thereby increasing the post-treatment costs of sludge. As a matter of fact, it has been noted that the treatment of sewage sludge accounts for 60% of the overall cost of wastewater treatment. Although it has been suggested that sludge must be recycled for land applications, it is restricted owing to the health risk associated...
with it. This means that the majority of the produced sludge ends up being incinerated. The incineration process generates ash, which tends to go into landfills as it cannot be disposed of elsewhere due to the high heavy metal content and general toxicity. The rising costs and public sensitivity to sewage sludge disposal have provided considerable motivation to explore and develop strategies and technologies for the minimization of sludge production [3]. This has resulted in many scholars investigating other interesting alternative technologies such as adsorption [4,5] and biofilm processes.

The biofilm process has emerged as a cost-effective and environmentally friendly option to replace the sludge process. Thus, biofilm systems have been increasingly used in WWTPs, and this approach is said to address some of the drawbacks of using the sludge processes. Moreover, the use of biofilms has been noted to be an essential method for microorganisms to prevent external stress caused by chemical compounds that may result in the inhibition of biological processes. Additionally, the long start-up time for biofilm technology has also been reported as a major problem. Many mechanisms that biofilms use to protect themselves against chemical destruction have been reported, such as extracellular polymeric substances (EPSs), SOS, and quorum sensing [6]. These defense mechanisms of biofilms make them ideal for WWTPs, since wastewater is composed of different chemical compounds that are toxic to biological processes.

Biofilms are especially essential for sensitive processes such as SND. Many strategies have been investigated to accelerate the biofilm’s adhesion to the surface, thereby reducing the start-up time. Different studies have investigated various reactors for their ability to enhance biofilm formation, although several bioreactors have been shown to efficiently enhance biofilms in WWTPs, thereby enhancing the removal of inorganic materials. Their application is still limited in large-scale applications owing to the large capital costs associated with commissioning these reactors. Moreover, different chemicals such as nanoparticles have been investigated for their ability to enhance the biofilm’s attachment, e.g., ferric citrate [7] and ferrous oxalate [8]; however, the application of these methods is still limited in wastewater treatment, owing to high costs of applying them in WWTPs. Furthermore, there is limited research on the impacts of these chemicals on the microbial community structure, and the diversity of biofilms, particularly in the context of wastewater treatment, which makes them difficult to use in large-scale wastewater treatment processes. Thus, it is imperative to investigate cost-effective options to enhance biofilm formation.

Tian et al. [9] proved that pre-seeding with an existing biofilm improved the biofilm formation, thereby improving the NH$_4^+$-N removal. Wang et al. [10] also proved that dosing the reactor with acylated homoserine lactones (AHLs) and exogenous quorum-sensing substances improved the biofilm’s thickness. Furthermore, the benefits of using biofilms in WWTPs are widely studied. However, there are still limited reports on the ways of exploiting the biofilm’s properties to enhance the removal of inorganic materials such as total nitrogen (TN) and phosphorus. Hence, this paper reviews the different ways that the biofilm’s characteristics can be exploited to promote the maximum removal of inorganic materials.

2. Biological Wastewater Treatment and Emerging Research

Although various contaminants have been proven to be treatable by biological methods, such as cyanide and polycyclic aromatic hydrocarbons (PAHs), this review focuses on the biological removal of total nitrogen and phosphorus.

2.1. Total Nitrogen Removal

Total nitrogen is one of the contaminants of concern, which is treated by nitrification and denitrification. Total nitrogen accumulates in the environment from different industrial activities. It enters the water streams through several pathways, such as in runoffs from contaminated soil and agricultural fields. If nitrogen is left untreated, it can result in environmental damage and can pose threats to human health [11,12]. The aerobic nitrification and anaerobic denitrification approaches use microorganisms in a two-step process, whereby the first step involves the oxidation of ammonium to nitrate in aerobic
conditions, and the second step involves the reduction of nitrate to molecular nitrogen under anaerobic conditions [13].

Conventional nitrification and heterotrophic denitrification are carried out in separate reactors by two distinct groups of microorganisms, namely autotrophs and heterotrophs, respectively. Heterotrophic denitrification uses inorganic carbon as an electron donor [14]. The use of separate reactors increases the operational costs of traditional nitrification and heterotrophic denitrification processes. However, some researchers have proven that the microorganisms responsible for nitrification and denitrification can co-exist in the same reactor. This process is known as simultaneous nitrification and denitrification (SND) [15,16]. In this process, the different microorganisms responsible for denitrification and nitrification synergistically interact with each other. Although the mechanism of SND has not yet been studied comprehensively, some studies have reported that SND results from the formation of biofilms, for which anaerobic conditions are provided in the middle zone of the biofilm, while the nitrifiers reside in the outer zone of the biofilm (Figure 1) [17]. SND has received attention because it addresses all of the shortfalls of traditional nitrification and heterotrophic denitrification and has high nitrogen removal efficiency, reducing up to 16% of the eutrophication potential of WWTPs and 1.72 kg of greenhouse gas (GHG) emissions, which is equivalent to USD 0.303/d·m$^3$ savings in cost. Moreover, SND can also reduce the aeration costs by 50%. Therefore, SND has been deemed appropriate for the treatment of side steams and domestic wastewater feeds [18]. However, it has been reported that SND could induce competition between autotrophic anammox species and heterotrophic denitrifiers.

**Figure 1.** Schematic diagram of a biofilm immobilized onto the surface.

The denitrification reaction is thermodynamically more feasible than nitrification due to its sensitivity, the low-growth yield of anammox species, and the standard free energy ($\Delta G^\circ$) of the denitrification reaction compared to the anammox reaction [19]. Although SND is an important part of wastewater treatment, the microorganism responsible for these processes is slow-growing, meaning these processes are susceptible to inhibition by heavy metal loadings. Different studies have investigated methods of bypassing this inhibition, with different studies suggesting that the inhibition in biofilm systems is less severe compared to planktonic-based systems. This has led to many studies investigating different methods of enhancing biofilms for biological WWT [20]. While SND has been intensely investigated, there are still gaps such as a lack of studies investigating the enhancement of anammox bacteria to give them a competitive advantage in the SND system in order to achieve maximum nitrogen removal.
2.2. Biological Phosphorus Removal

Phosphorus accumulation in the environment can result in eutrophication in aquatic water systems, meaning it is crucial to remove it from WWTPs [21]. Phosphorus enters the wastewater treatment plant through residential water that contains a large amount of phosphorus from the use of synthetic detergents that contain high polyphosphate concentrations. Orthophosphates and polyphosphates are the most common forms of phosphorus found in WWTPs, which constitute 70 to 90% of the phosphorus found in WWTPs. The rest are from inorganic compounds.

Biological phosphorus removal is a sustainable and environmentally friendly method to reduce phosphorus from WWTPs [22]. Traditional biological phosphorus removal involves the use of phosphate-accumulating organisms (PAOs). This process involves the circulation of activated sludge between the anaerobic and aerobic phases. During the introduction of the effluent water into the anaerobic phase, the abundance of degradable carbon sources such as volatile fatty acids (VFAs) present in the sludge induces the uptake of this acid by phosphate-removing bacteria, thereby releasing phosphate into an aqueous solution, while phosphorus uptake occurs in the aerobic phase. It has been shown that wasting excess sludge results in high phosphorus removal [23].

Recently, it has been shown that denitrification can be achieved by denitrifying PAOs (DPAOs) under anaerobic conditions. This occurs when the PAO ingests high amounts of phosphorus, thereby promoting the uptake of nitrate as a final electron [24]. Wu et al. [25] successfully developed an innovative denitrifying phosphorus removal and partial denitrification anammox process in an ABR-CSTR reactor to improve the nitrogen and phosphorus removal. The author indicated that this process successfully removed up to 97.57% and 95.66% of total inorganic nitrogen and phosphorus, respectively. These results are comparable to the result obtained by Wang et al. [26], who obtained 78.35% and 98.34% removal rates for nitrate-to-nitrite transformation and phosphorus using a novel denitrifying nitrite accumulation and phosphorus removal (DNAPR) process. DPAOs are said to produce 40% less energy as compared to PAOs, meaning DPAOs requires less COD and aeration. This makes DPAOs ideal over PAOs, particularly for the treatment of domestic wastewater, which contains low levels of COD [27]. It has been suggested that more COD could be saved if DPAOs could be used for SND via nitrite. Thus, there has been a rising interest in applying DPAOs for SND.

3. Role of Biofilms in the Biological Wastewater Treatment System

The formation of a biofilm is a complex process that is influenced by the conditions of the surroundings, such as the properties of the surface and the deposition of the organic materials in the surface carrier. Before the microorganism attaches to the surface for biofilm formation, the surface will initially be acclimatized to adsorb organic molecules present in the surrounding environment. This step is followed by the attachment, adhesion, retention, and proliferation of the microorganisms to the surface (Figure 2). The motile bacteria, which have flagella and pili, are responsible for initiating biofilm adhesion to the carrier material. The flagella is essential in the movement of bacteria from the planktonic state to the surface.

Type IV pili are filamentous protein complexes that have been known to be responsible for the initial adhesion of the cells to the surface. They have also been said to be vital for the maturation of biofilms [6]. The chemical properties of the surface affect the absorption and distribution of the surrounding organic material into the carrier surface. Moreover, the type of organic materials absorbed by the surface may also change the properties of the surface carrier. Biofilms usually grow in a consortium instead of a monoculture. During the biofilm formation, the microorganisms of the same family communicate by transmitting chemical compounds that signal to the planktonic species to migrate to the biofilm state. This microbial communication is known as quorum sensing [28]. As microorganisms form biofilms, they produce EPSs, which act as a protective layer for the biomass and helps them
to attach to the surfaces. Biofilms are generally resistant to killing by external contaminants, as opposed to planktonic bacterial cells.

Figure 2. Formation and maturation of a biofilm from the planktonic state.

The resistance of a biofilm towards external stress is associated with several factors, such as the changes in gene expression in response to biofilm growth and the external stresses and intrinsic properties of the biofilm’s structures [4]. Biofilms in wastewater treatment are often exposed to inhibitory chemicals. It has been hypothesized that the exposure of a biofilm to inhibitory chemicals may induce biofilm formation. This hypothesis has been tested in various studies, where *Pseudomonas aeruginosa* and *Escherichia coli* (*E. coli*) were found to be induced by aminoglycoside antibiotics and a *Mycobacterium avium* biofilm was induced by hydrogen peroxide [29]. The products of this phenomenon were named stress-inducible biofilms.

Biofilms play a significant role in the removal of chemical and microbiological contaminants in WWTPs. They are used in various reactor setups, such as moving bed reactors (MBRs) [30], trickling filters, and rotating contactors [31]. The use of biologically active carbon (BAC) is one method used for the generation of naturally occurring biofilms. Generally, granular activated carbon is used in WWTPs as a filter to remove undesirable microbes and organic and inorganic contaminants. However, the microbes tend to colonize the rough porous surfaces of the carbon, thereby growing into biofilms, which can then filter the contaminants in wastewater. The biofilm-based wastewater treatment systems are preferred over sludge processes owing to their benefits, which include a minimum requirement for space, low hydraulic retention time, high concentrations of active biomass, and low production of sludge [32]. Additionally, the microorganisms in the biofilm system tend to be more diverse as compared to those present in the sludge system, making biofilms capable of treating a diverse range of organic pollutants.

The performance of the biofilm greatly depends on its formation. The formation of the biofilm is the result of biotic and abiotic factors that include (1) the diversity of the microbial community, (2) the physical properties of the surface carrier, which are linked to the interaction between electrostatic and surface energies in the microbial adhesion to the carrier surface, (3) the roughness of the surface, (4) the chemical properties of the surface carrier, and (5) factors such as the temperature and pH. The most crucial factors in the
formation of biofilms have been said to be the physical and chemical properties of the carrier surface. The use of inorganic materials as carriers has been shown to cause large resistance, poor permeability, and low formation of biofilms as compared to carriers that are modified, e.g., modified zeolites or light porous ceramic carriers. Organic-based carriers are better options for biofilm formation. Moreover, the carriers that are efficient in promoting biofilm formation are those that can also promote microbial growth by serving as electron donors. The hydrophilicity and electronegativity of the carrier have also been noted to play a crucial role in the formation of biofilms [32].

4. Factors Hindering the Application of Biological Processes and the Role of Biofilms in Mitigating Them

The ammonium-oxidizing bacteria (AOB) are sensitive, and they have been reported to be prone to inhibition by various compounds present in wastewater, thereby inhibiting nitrification [33]. High concentrations of ammonium and phenolic compounds have been known to have an inhibitory effect on SND. These compounds are normally found in wastewater effluents from many industrial processes, such as petrochemical manufacturing, coke production, landfill leachate, and petroleum refinement [34]. Kim et al. [35] reported the inhibition of nitrification using 200 mg/L of thiocyanate, 0.2 mg/L of free cyanide, 200 mg/L of phenol, and 100 mg/L of µ-cresol. There can also be selective inhibition, such as the inhibition caused by free-ammonia or toxic compounds. This type of inhibition only inhibits nitrite-oxidizing bacteria (NOB) but has a minimal inhibition activity towards AOB. Additionally, saline water also has a high inhibitory effect on nitrite-oxidizing bacteria (NOB); it can cause plasmolysis and the loss of activity of NOB [36].

The presence of free ammonia and free nitrous acid can also inhibit SND. The first step of SND is known to be sensitive to high concentrations of free nitrous acid (FNA), while the second step is sensitive to high free ammonia (FA) concentrations. It has been reported that Nitrobacter species are inhibited by 0.1–1.0 mg/L of ammonia, while ammonia-oxidizing species are inhibited by 10–150 mg/L of ammonia [37,38]. Qian et al. [39] reported that NOB decreased by 29.2% while AOB decreased by 15.9% in the presence of FA. Additionally, Zhang et al. [40] also reported the inhibition of NOB by 36.06–50.66 mg/L of free ammonia. These results are comparable to the results obtained by Li et al. [41], who reported the inhibition of NOB by high FA concentrations. However, Bhattacharya and Mazumder [42] claimed that 40 mg/L of free ammonia did not inhibit the nitrite oxidation when a pre-acclimatized biomass was used, while 3.5 mg/L of free ammonia completely inhibited nitrite oxidation when a non-acclimatized biomass was used. SND is beneficial over traditional nitrification and subsequent aerobic denitrification because it addresses some of the drawbacks of the traditional nitrification subsequent aerobic denitrification process, such as the treatment of low-C/N wastewater. Municipal wastewater tends to have a low carbon-to-nitrogen ratio (C/N), which may necessitate the addition of a carbon source to provide electron donors for denitrification thus, preventing a decline in the nitrogen removal efficiency [17].

Some heavy metals are important in trace amounts; however, they can be toxic when they are present in large concentrations. Among the toxic heavy metals, arsenic, cadmium, mercury, and lead have been reported to be in the top ten major toxic heavy metals that affect the biological wastewater treatment. Additionally, these heavy metals have been shown to inhibit some microbial processes that are important to different biogeochemical cycles. The increased heavy metal concentration causes a decrease in microbial respiration, leading to the inhibition of biological processes. Nitrification and the biological phosphorus removal process are among the biological processes that are heavily inhibited by high heavy metal concentrations [33]. Several studies have investigated the nitrification effects of different heavy metals, with some studies reporting inhibition thresholds of 25.0 mg/L for cadmium [43], 0.5 mg/L for chromium [44], 0.5 mg/L for copper [45], 1000 mg/L for lead [46], 0.2–4.0 mg/L for nickel [45], and 0.1–10 mg/L for zinc [46]. PAOs have also been
reported to be inhibited by organic substrates, nitrite or free nitrous acid (FNA), nitrate, ammonium or free ammonia (FA), and other heavy metals.

Biofilms are essential in WWTPs, since the wastewater may contain a hazardous compound that negatively affects the biological wastewater treatment systems. The impact of external contaminants on biofilm-mediated biological wastewater treatment systems is not as severe as on non-biofilm-mediated systems. The microorganisms that aggregate in the biofilm structure are protected from chemical stresses; thus, biofilms are said to have resistance to external stress. This high-stress resistance makes these biofilm structures especially important in sensitive biological processes such as SND [6, 47]. It has been noted that biofilm cells are 10- to 1000-fold less prone to being killed as compared to planktonic bacteria. This is due to several factors that are associated with the defense mechanisms of biofilm possess. Among the defense mechanisms, EPSs represent the main defense mechanism against eternal stresses for biofilms.

5. Approaches to Improve Biofilm-Based Wastewater Treatment

Enhancing the biofilm formation is important in biofilm-based systems, as this improves the biological wastewater treatment system. The following section will discuss the different approaches used to enhance the biofilm formation. Moreover, other approaches that can be exploited will be suggested.

5.1. Biofilm Reactors

Different bioreactors have been used to promote biofilm-based wastewater treatment (Figure 3). These biofilm-based systems are especially important for processes that require different conditions, such as aerobic and anaerobic conditions. Moreover, the biofilm systems are important to prevent the washout of the bacteria used in the biological wastewater treatment. The washout is normally caused by the slow growth of microorganisms responsible for biological processes. Nitrifying bacteria are typical examples of microorganisms that are prone to washout. The biofilm systems prevent washout by maintaining the density of the bacteria in continuous systems [48]. The biofilms can provide aerobic and anaerobic conditions for different microorganisms in the bioreactor.

In general, biofilm reactors are designed to address the issue of low biomass concentrations and long hydraulic retention times experienced during the sludge process. The microbes in biofilms tend to compete for substrates such as ammonia, organic carbon, and oxygen. Those microbes that consume organic carbon and oxygen, such as nitrifying bacteria, are normally found on the outer layers where the concentration of oxygen is high, while those bacteria that require low concentrations of oxygen, such as denitrifying bacteria and DPAO, exist in the inner layer of the biofilm. Hence, biofilm reactors are common for SND in WWTPs.

Membrane-aerated biofilm reactors (MABRs) are well-studied biofilm reactors for wastewater treatment. In MABRs, the biofilm aggregates on the outer side of the membrane while it is aerated through the inner side of the membrane [49]. The operation of an MABR is flexible, especially when it comes to the diffusion of a gas or liquid through the membrane lumen. The ability to provide exogenous electron acceptors or donors through the membrane lumen makes such reactors suitable for treating difficult wastewater. Additionally, this type of reactor is suitable for the treatment of low carbon-to-nitrogen ratio (C/N) wastewater. This is done by supplying the methanol or hydrogen into the reactor through the membrane lumen, which further stimulates the biological wastewater treatment [50].
Other biofilm reactors such as packed bed and fluidized bed biofilm reactors have been investigated for the treatment of wastewater. Fluidized bed reactors have been shown to have high rates and low pressure drops as opposed to packed bed biofilm reactors. Moreover, the fluidized bed reactors have been reported to have no bed clogging and lower external transport resistance. These two reactors have been specially investigated for their applications in SND. These reactors support the existence of the aerobic and anoxic conditions that are required for SND. However, it has been noted that the existence of different microorganisms that compete for substrates necessitates the proper control of the ratios of organic carbon to ammonia in this type of bioreactor [48].

Other reactors that have been studied for SND include sequencing batch reactors (SBRs), moving bed biofilm reactors (MBBRs), sequencing batch biofilm reactors (SBBRs), moving biofilm reactors, and oxidation ditch reactors. SBBRs are highly recommended for SND over all other reactors mentioned, especially for the treatment of high-strength industrial wastewater. Furthermore, the SND–SBBR approach is ideal, since it saves on construction and energy costs, has low oxygen demands, and does not require expensive fillers and dosages. The reduced requirement for oxygen in SBBRs could potentially reduce the concentration of NO\(_x^-\) (NO\(_2^-\) and NO\(_3^-\)), thereby reducing the carbon required for denitrifying bacteria. This could result in extra carbon sources being accessible for denitrifying bacteria. This is why SND has been said to reduce 40% of the carbon needed for denitrification and to increase the rate of denitrification by up to 63% [51]. MBBRs have also been reported to be a suitable reactor for SND. The types of carriers used in MBBRs have been intensely investigated, with different studies reporting different suitable carriers [52]. However, biofilms have been said to cause fouling in MBBRs. Table 1 compares the performances of different bioreactors.
Table 1. Comparison of the performances of different biofilm reactors for the treatment of nitrogen and ammonium.

| Reactor                          | Contaminant                  | Carrier Types         | Removal Efficiency | Influent  | Effluent          | Ref                      |
|----------------------------------|------------------------------|-----------------------|--------------------|-----------|-------------------|--------------------------|
| hybrid aerated biofilm reactor   | Total nitrogen and ammonium | nylon biomass carrier | 96.61% for NH$_4^+$-N, 72.23% for TN | -         | 2 mg/L for NH$_4^+$-N, 72.23% for TN | Chang et al. [53]         |
| packed bed                       | Total nitrogen               | K1 carriers           | 84.8 ± 4.3%        | 207.6 ± 29.9 mg N/L | 876.8 mg N/(L·d)      | Li et al. [54]            |
| aerobic fluidized bed biofilm reactor | Total nitrogen and ammonium | modified polyurethane sponge | TN, 66.98 ± 4.23% NH$_4^+$-N, 74.70 ± 2.30% | -         | -                 | Ren et al. [55]           |
| sequencing batch biofilm reactor | TN removal efficiency       | Resin fillers         | 93%                | 68 mg/L   | -                 | Lu et al. [56]            |
| moving bed                       | Total nitrogen               | noxKaldness-K3        | 96.2%              | 64.3 to 68 mg-N/L | -                 | Almomani and Bohsale. [57]|
| sequencing batch                 | Total nitrogen and ammonium | polypropylene multifaceted hollow balls | NH$_4^+$-N (95%), TN (88%) | 50 mg/L NH$_4^+$-N, 50 mg/L nitrate-N | 6 mg/L for NH$_4^+$-N | Hong et al. [58]          |

5.2. Modification of Carriers to Promote Biofilm Adhesion

Methods to enhance the biofilm’s attachment and stability have gained in popularity, to the extent that there has been a rise in studies investigating good carriers that support biofilm growth. The type of carrier used has been said to play a major role in the bioreactor’s performance, especially in MBBR systems. The common materials used for biocarriers include high-density polyethylene, polypropylene, and polyethylene/polymeric-reticulated sponge or foam. The most commonly used biocarriers in MBBRs are small cylindrical-shaped polystyrene carriers, LEVAPOR carriers, polyethylene cylinders, and polymeric carriers (polyhydroxybutyrate/polycaprolacton) (Table 2). These biocarriers can be found in different sizes, with densities ranging 0.94–0.98 g/cm$^3$ [59]. Some researchers have been investing time in investigating carriers that will support different biofilm thicknesses.

The type of substrate used has also been reported to play an important role in the attachment of bacteria to the surface. The perfect carrier must cause turbulence to transport dissolved oxygen and nutrients to the bacteria that are attached. Additionally, a good carrier is said to possess a positive surface charge, since the bacteria have a negative surface charge (Figure 4). The use of inappropriate carriers may disturb the microbial growth, resulting in unsteady bioremediation [60]. Hence, the effect of the surface chemistry for MBBR carriers in biofilm formation has been intensely investigated. There has been an increase in studies investigating ways to promote the attachment of bacteria to the substratum. The modification of carriers or carrier materials is a popular method of improving the biofilm attachment, as well as maintaining a great thickness of the biofilm. Some of the methods used for surface modification include pre-seeded biofilms, film coatings, and physical or chemical changes to the surfaces. Different carriers such as granular-activated carbon, polyurethane materials, polyethylene plastics, polyurethane foam, polymeric gels, and polyurethane sponges have been shown to enhance the biofilm attachment, e.g. polyurethane sponge carriers have been reported to enhance the immobilization as a result of their high porosity [61].
Table 2. Popular carriers and their characteristics [59].

| Brands Name | Carrier Materials | Specific Surface Area (m²/m³) | Dimensions (Depth mm × Diameter mm)/(L × W × D) |
|-------------|-------------------|-------------------------------|-----------------------------------------------|
| AnoxKaldnes™ K3, | polyethylene       | 500, 500                      | 7.2 × 9.1, 10 × 25                             |
| AnoxKaldnes™ K1, | polyethylene       | 500, 500                      | 7.2 × 9.1, 10 × 25                             |
| K5, Biofilm Chip P, Activecell™ 450 | polyethylene | 800, 900, 402                  | 3.5 × 25, 3 × 45, 21 × 16                      |
| AnoxKaldnes™ K2 | polyethylene       | 350                           | 15 × 15                                       |
| AnoxKaldness™ Natrix C2 | polyethylene | 220                           | 30 × 36                                       |
| AnoxKaldness™ Biofilm Chip M | polyethylene | 1200                          |                                                |
| AnoxKaldnes™ Z200, Z400 | polyethylene | –                             | 30 mm diameter, 200 μm (thickness), 30 mm diameter, 400 μm (thickness) |
| LINPOR | polyurathane cubes | 270                           | 15 × 15 × 15                                 |
| LEVAPOR | soft porous foam cubes | 20,000                        | 14 × 14 × 10–20 × 20 × 10                     |
| polypropylene multifaceted hollow balls | polypropylene | -                             | 25 mm diameter                                 |
| ABC4™, ABC5™, ActiveCell™ 515 | plastic | 600, 660, 515                  | 14 × 14, 12 × 12, 15 × 22                     |
| BWT15™, BWTX™, Bioportz™, CM-10DTM, ActiveCell™ 920 | plastic | 828, 640, 589, 750, 680       | 15 × 15 × 5, 15 × 15 × 10, 14 × 18, 9 × 13, 15 × 15 × 10 |
| Biosphere N, Spira 12, 14, FLOCOR-RMP, AnoxKaldness™ Natrix M2, Natrix F3 | polyethylene | 800, 650, 600, 260, 220, 200 | 9 × 13, 12 × 12, 14 × 14, 10 × 16, 50 × 64, 37 × 46 |
| Polyurethane sponge | modified polyurethane sponge | 32,000                        | 10,000 × 10,000                               |

Figure 4. Interaction between the carrier surface and bacterial cell surface: (A) electrostatic interaction between the cell surface and the carrier surface; (B) hydrogen bond formation between the bacterial cell surface and carrier surface [32].
Shitu et al. [8] reported 98.86 ± 0.7% ammonium removal under 35% saline conditions when modified sponge biocarriers (SB) were used in MBBRs. Moreover, the modified biocarriers were said to promote rapid microbial growth. Ghahramani et al. [62] also investigated the use of polyvinylidene fluoride (PVDF) foam carriers in MBBRs. The results showed that these carriers promoted biofilm growth while providing great biofilm protection from MBBRs, achieving 77% ± 7% organic removal efficiency. An electrically bound biofilm reactor was also used to enhance the attachment of biofilms. Srinivasan et al. [63] reported a 79.3% biodegradation rate within 28 h and enhanced biofilm attachment by using an electrically bound biofilm reactor with an anode (nematic liquid crystal electrode) and three different cathodes (Al, Cu, and Pt).

5.3. Enhanced Adhesion by Using Biofilm as a Substratum

It has also been shown that a pre-established biofilm can also act as a good substratum surface modification method that efficiently facilitates the development of more biofilms. The pre-established biofilm facilitates the development of an additional biofilm through modification of the substratum surface and the water by attracting planktonic cells into the pre-existing EPSs. This approach has been shown to result in faster bacterial attachment to the carrier surfaces. Moreover, some studies have shown that the coating of carriers to increase the roughness enhanced the attachment of the biofilm [64].

5.4. Enhanced Biofilm Adhesion and Performance by Exploiting EPSs

There are different properties of biofilms that are currently exploited for the enhancement of biofilm technology in WWTPs, such as EPSs. The compositions of EPSs differ depending on the medium that the biomass grows into. The EPS is normally made up of exopolysaccharides, DNA, and proteins. The microorganisms that grow in the center of the biofilm tend to be the microorganisms that are anaerobic, while the ones that grow on the outer surface are aerobic microorganisms [65]. The biofilm is normally enhanced by modifying the surfaces such that they are Ideal for biomass attachment. When the microbes grow into a biofilm, they produce a complex high-molecular-weight protective layer known as an EPS. Microorganisms in biological wastewater treatment grow as biofilms, sludge flocs, and granules, and are protected by an EPS that forms a three-dimensional defensive layer against external stresses. Usually, the EPS forms 50–80% of the overall biomass weight. The EPS is a mixture of polymers, made up of polysaccharides, uronic acids, nucleic acids, proteins, humic acids, and lipids. The EPS accumulates through different mechanisms, comprising excretion, secretion, cell lysis, and sorption [66].

EPSs have been shown to enhance biofilms; thus, some studies have used them as coatings for carriers to improve the biofilm attachment, thereby enhancing the efficiency of the biological wastewater treatment [6]. The film coating of carriers has been carried out using molecules that are similar to EPS molecules, such as glucuronic acid. Dextran has also been shown to be great for coating, particularly for the promotion of nitrifying bacteria such as Bacillus subtilis. Pre-seeding has also been said to enhance the attachment of certain anammox microorganisms. Furthermore, the carrier topography has been shown to play an important role in biofilm adhesion and formation. Thus, there have been researchers that have investigated the use of rough materials to coat carriers. The materials such as activated carbon, silica, and charcoal have been shown to increase the surface area for the attachment of anammox bacteria [9].

5.5. Enhanced Biofilm Performance by Exploiting Quorum Sensing

Quorum sensing has also been shown to be an essential component of biofilms, and it can be exploited in several ways to enhance biological wastewater treatment. Quorum sensing has been said to enhance the attachment and production of EPSs, which are essential aspects in the protection of the biofilm [67]. Quorum sensing is a form of communication among bacteria that occurs through the production of chemical signals and autoinducers (AIs). Some of the AIs responsible for the biofilm communication include acylated homoser-
ine lactones (AHLs), autoinducing peptides (AIPs), autoinducers-2 (AI-2), pseudomonas quinolone signal (PQS), and autoinducers-3 (AI-3). However, the most common AIs are AHLs, which are controlled by Luxl/LuxR systems. These AIs are produced by a wide range of bacteria and have been reported in over 100 species of Proteobacteria, including 25 Gram-negative bacterial species [68]. Wang et al. [69] investigated the role of AHLs in biofilm adhesion and found that AHLs significantly improve bacterial adhesion as well as biomass production, with maximum increases of 2.26, 2.36, 2.52, and 2.80 times in four different biofilms. The author concluded that the observed increases were due to the long-chain AHLs, which resulted in stronger hydrophobicity and hydrolysis resistance.

Quorum auto-inducer AHLs have been shown to play an important role in biological bioreactor systems. AHLs have been reported in different wastewater treatment bioreactors with large quantities of microbial consortia growing as flocs, granules, or biofilms. The dosing of AHLs has been known to affect the performance of the reactor, as well as the properties of the activated sludge. AHLs have been said to help in preventing biofouling of the bioreactors. The presence of AHLs induces acylase, which is one of the many AHL-hydrolyzing enzymes that degrade AHLs. This hydrolysis process of AHLs by acylase is exploited for the prevention of biofouling in bioreactors [70]. Yeon et al. [71] successfully proved the biofouling prevention of MBRs via the activation of AHLs with porcine kidney acylase I. The author further noted that quorum sensing could potentially be an innovative solution to control reactor biofouling in WWTPs. It has been noted that the addition of AHLs to activated sludge increases the rate of ammonia oxidation as well as the expression of amoA genes by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). Although quorum sensing is beneficial to the biological wastewater treatment, more studies need to be conducted to understand the impacts of the different toxic chemical compounds present in WWTPs. AIPs have not received much attention in the context of wastewater treatment, although they have been shown to enhance attachment through accessory gene regulators [72].

5.6. Enhanced Biofilm Performance by Exploiting SOS

Another defense mechanism is SOS. It has been intensely studied in the medical field, and it was found that SOS is the main cause of antibiotic resistance by biofilms. SOS is the microbial response system to DNA damage due to the expression of antibiotic resistance genes in bacteria that are in the biofilm [47,73]. However, SOS has not yet been explored in the context of wastewater treatment. This stress-inducible biofilm has been reported to occur as a result of the induction of SOS when the biofilm experiences stress. SOS is induced as an emergency response to the threat. Chemicals that inhibit microbial growth may induce SOS. When the bacteria are exposed to external stresses, normal cell division is inhibited following DNA damage.

The SOS phenomena have been studied in an E. coli biofilm, for which SOS was induced by exposing the biofilm to UV light. When microorganisms growing in biofilms are exposed to a compound that damages DNA, the genes that control SOS are induced by the SOS repressor locus for X-ray sensitivity, involving LexA and sensor/inducer recombinase A (RecA). These DNA repair genes are responsible for repairing damaged DNA [73]. They do this by binding the single-strand DNA to RecA, thereby activating it. This results in the formation of the nucleoprotein filament, which promotes the self-cleavage of LexA, thereby leading to the de-repression of genes responsible for SOS. The helicase unwinds the double-strand DNA, increasing the single-strand DNA. This, in turn, induces an SOS response. This process occurs when a DNA polymerase stalls at a lesion. The first induction of SOS normally involves high-fidelity repair mechanisms, followed by low-fidelity mechanisms; this is because the SOS response is temporally controlled. The several DNA polymerases involved in the damage tolerance pathway, namely PolII (polB), PolIV (dinB), and PolV (umuC, umuD), are some of the DNA polymerases that remain active even after great DNA damage. They are capable of repairing irreparable DNA lesions that could block DNA
replication. SOS can also be induced by exogenous and endogenous triggers, conjugative plasmid DNA transfer, and transformation [74].

It has been reported that the SOS in *Clostridia* is induced by methyl methanesulfonate and UV radiation. Moreover, the induction of SOS in *Pseudomonas Aeruginosa* has been shown to promote biofilm formation [75]. The SOS phenomena can be used to improve the attachment of bacteria. SOS can be exploited to benefit biological wastewater treatment. This can be done via the induction of SOS, such that the microorganism in the biofilm can induce an emergency response to toxic compounds entering WWTPs, thereby inducing their defense mechanism and mitigating the inhibitory effects of toxic compounds on biological processes in WWTPs [47]. It has been noted that the SOS increases the adhesion and is also responsible for the formation of biofilms. Additionally, other studies have reported that SOS is induced by the attachment of bacteria to the surfaces; hence, bacterial adhesion through the flagella increases when SOS occurs [76]. Table 3 compares the effects of different enhancement methods on the biofilm formation.

### Table 3. Comparison of different methods used to enhance biofilm formation.

| Ref            | Method of Biofilm Enhancement | Mature Biofilm | Biofilm Growth Rate | Full Carrier Coverage | Biofilm Thickness          |
|----------------|-------------------------------|----------------|--------------------|-----------------------|---------------------------|
| Tian et al. [9]| silica-functionalized carriers | day 106        | 1.0 µm/d           | days 32 and 57        | 56.2 ± 8.3 µm on day 32, 82.6 ± 10.2 µm on day 57 |
| Tian et al. [9]| dextran-functionalized carriers | -              | 0.7 µm/d           | days 32 and 57        | 58.1 ± 8.9 µm on day 32, 71.5 ± 8.4 µm on day 57 |
| Tian et al. [9]| Biofilm pre-seeding            | -              | -                  | days 32 and 57        | 56.2 ± 8.3 µm on day 32, 78.5 ± 9.3 µm on day 57 |
| Wang et al. [10]| AHLs dose                      | 24 h           | -                  | -                     | 33.56 ± 0.29 µm           |
| Wang et al. [10]| Acylase dose               | -              | -                  | -                     | 5.71 ± 0.11 µm           |
| Li et al. [54]| Non-modified biocarriers      | day 100        | -                  | day 35                | 182.5 ± 44.1 µm          |

A study conducted by Tian et al. [9] compared silica functionalization (roughness enhancement), dextran functionalization (chemical enhancement), and biofilm pre-seeding (pre-biofilm enhancement) approaches to enhance biofilm formation. The results obtained from this study indicated that the silica functionalization and pre-seeding of carriers with denitrifying bacteria improved the rate of NH$_4^+$-N degradation when compared to virgin carriers. Moreover, the dextran-functionalized carrier did not decrease the start-up period. Additionally, Xu et al. [77] showed that the presence of EPS improved the initial attachment of the biofilm. Moreover, Wang et al. [10] indicated that dosing with AHLs at 0.5-, 1-, and 2-fold volumes resulted in improvements in the thickness of the biofilm from 10.97 ± 0.34 to 16.12 ± 0.21 µm (46.95%), 27.44 ± 0.17 µm (150.14%), and 33.56 ± 0.29 µm (205.93%) after 24 h, respectively. These results clearly show that the addition of exogenous AHLs stimulated the secretion of AHLs by quorum sensing bacteria, thereby improving the adhesion. Moreover, the addition of the two-fold volume of external AHLs was shown to reduce the reversible adhesion time from 780 min to 60 min.

### 6. Factors Affecting the Application of Biofilm Characteristics to Enhance Biofilm Formation

The type and characteristics of the carrier used affect the attachment of the biofilm. Carriers generally have a negatively charged surface, which makes it difficult for negatively charged bacteria to attach. Additionally, bacteria prefer to attach on rougher, hydrophobic surfaces coated by films. The adhesion of bacteria to the surface can be increased by increasing the interface fluid velocity. The pH has also been shown to affect the surface charge of the bacteria. Higher liquid-phase pH values have been said to cause electronegativity of the bacterial surface as a result of amino acid ozonation. Therefore, pH variations change
the electrical behavior of the bacterial surface, thereby affecting the adhesion of the bacteria to the carriers [59].

Quorum sensing plays a crucial role in biofilm formation. AHLs are some of the autoinducers produced for quorum sensing [78]. Although these autoinducers are important in biofilm formation, their presence in wastewater induces the synthesis of acylase, which is an AHL-degrading enzyme [72]. The presence of this enzyme affects the quorum sensing, thereby affecting the EPS production and subsequently affecting the biofilm formation. Wang et al. [10] reported that dosing wastewater with acylase reduced the biofilm thickness by 47.95% from 10.97 ± 0.34 to 5.71 ± 0.11 µm in municipal wastewater, while a decrease of 63.38% from 13.57 ± 0.21 to 4.97 ± 0.10 µm was observed in industrial wastewater. Additionally, c-di-GMP plays a crucial role in the biofilm’s formation and its three-dimensional structure. Moreover, c-di-GMP controls the swimming, swarming, and twitching processes and regulates the production of EPS and extracellular DNA, which are essential for the initial attachment of the bacteria to the surface [79]. It has been shown that high levels of EPS and c-di-GMP can coexist [80], although c-di-GMP plays an important role in biofilm formation. High levels of c-di-GMP are linked with reversible attachment and detachment of the biofilm. Increases in c-di-GMP have been associated with activation of the BalA protein, which has been shown to be responsible for biofilm dispersal in Pseudomonas aeruginosa. Although c-di-GMP promotes the production of EPS, it can be detrimental to quorum-sensing-dependent biofilm formation [78,81]. While using the biofilm’s properties to enhance biofilm performance seems to be a promising strategy, there is still a lot of work that needs to be done to ensure that these techniques are efficient and will not affect the bacteria that are important in biological wastewater treatment.

7. Conclusions

Biofilms play an important role in the treatment of contaminated wastewater. Although biofilms play a crucial role in wastewater treatment, the properties of biofilms and their role in wastewater treatment are not widely researched. This study presented different ways that the properties of biofilms can be used to enhance biological wastewater treatment systems. The roles of EPS, SOS, and quorum sensing have been reviewed. Furthermore, the strategy of exploiting these properties to improve the biological wastewater treatment systems has also been highlighted. This review provides insight into how biofilms can be manipulated to prevent the inhibitory effects of toxic compounds entering the wastewater treatment plant, as well as providing a sustainable way of improving the performance of biofilm-based wastewater treatment systems. Although several studies have reported on the impact of the exogenous dosing of biofilm systems by EPS, SOS, and quorum sensing, there are still limited studies investigating their impacts on the microbial diversity, as well as their impacts on large-scale applications. Furthermore, optimization studies involving EPS-, SOS-, and quorum-sensing-enhanced biofilm systems are imperative.

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