# A review on the advances of nitrifying biofilm reactors and their removal rates in wastewater treatment

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**Abstract:**

Growing demand for efficient wastewater treatment systems leads to the development of new technologies. Biofilm-based reactors can be used for the treatment of a variety of wastewaters and these reactors are resistant against toxic environment. Bioreactors, such as sequencing batch biofilm, moving bed biofilm, and etc. are advanced techniques to treat various types of wastewaters with diverse operating conditions. Ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and Anammox (anaerobic ammonium oxidation) bacteria are reported to be responsible for nutrient removal. In recent decades, the performance of these systems is widely studied and compared for a number of wastewater treatment applications. In general, they are particularly suitable, for high-rate nitrification and nitrogen removal. The efficiency of these reactors has been confirmed in the laboratory and large-scale plants. Their efficiency depends on surface area of the biocarrier, the filling percent volume of biofilm carriers, organic loading and diffused aeration supply. 50% to 98% removal in chemical oxygen demand removal was reported for <12 h of hydraulic retention time, 0.2 mg/L to 6.5 mg/L dissolved oxygen concentration and temperature range of 15-35 °C. Also, the ratio of nitrate to ammonium conversion was from 0.2 to 90 and $N_2$ conversion was from 0 mg to 8.5 mg. This review

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studied each of these bioreactors in the removal of nutrients (N, P, and O) from different wastewaters and compared them to conventional treatment. The review also includes the relevant studies on laboratory and pilot scales bioreactors to enhance their performance and reduce their costs.

***Keywords:*** High ammonia wastewater, Biological reactor, Nitrification, Denitrification, Support media.

## 1. Introduction

Excess nitrogen loads have been recognized to be one of the serious causes which adversely affect the water quality [1].Worldwide, there is an effort to reduce the emissions of nitrogen compounds to the surface waters and the atmosphere [2]. Ammonia is the most abundant inorganic nitrogen in various wastes and wastewaters, such as municipal wastewater, landfill leachate and livestock waste [3]. Excessive discharge of ammonia into the water environment can cause toxicity to aquatic organisms and hence eutrophication [4]. When aquatic ecosystems experience increased nutrients, the phytoplanktons and other photosynthetic plants growth explosively, commonly known as algal blooms [5]. The algal blooms limit the amount of dissolved oxygen (DO) required for respiration by other animal and plant species in the water [6]. In extreme cases, the anaerobic conditions encourage the growth of bacteria that produce toxins. Algal blooms are highly toxic and once the water reaches the anaerobic conditions, the growth of more toxic bacteria is promoted. The consequence is extensive deterioration of water quality and a decline in the availability of clean drinking water [7].

Ammonia can be removed by using physical, chemical and biological methods [8], and sometimes in combinations of physical-chemical [9] or biological-chemical [10]. The cost-effectiveness of biological treatment has increased dramatically in the past few years since several processes for the biological removal of ammonia from wastewaters have become available [11]. For example, Rikmann *et al*, used undiluted reject stream from the dewatering stage of anaerobic sludge to start-up the autotrophic nitrogen removal in two pilot-scale configurations, nitritation-Anammox and deammonification. They observed that deammonification process produces up to 90% less excess sludge compared to denitrification and needs smaller process tanks which results in lower treatment costs [12]. Because biological nitrogen (N) removal is more effective and relatively inexpensive, it has been adopted as compared to the physicochemical processes [13]. Conventional biological N removal has been widely applied at full scale to treat wastewater [14], it is based on autotrophic nitrification and heterotrophic denitrification.

Nitrification (biological oxidation of ammonia to nitrate through nitrite) is an economical and sustainable means of ammonia removal as it eliminates the need for chemical addition [15]. The first nitrification step is N-$NO_2^-$ formation (1), and the second step (2) is N-$NO_3^-$ formation [16-18]

$$NH_4^+ + 1.5\ O_2 \longrightarrow NO_2^- + 2H^+ + H_2O \qquad (1)$$

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^- \qquad (2)$$

Nitrifiers are slow-growing microorganisms. Growth in suspension requires long residence times, or diluted feed streams (a situation frequently found in domestic wastewater treatment processes) [19-21]. In these cases, biofilms can represent an effective solution to successfully retain biomass in the reactors [22]. Several technologies based on the biofilm have been developed as alternatives to the traditional wastewater treatment [23, 24]. Biofilm reactors represent the primary means to harness the usefulness of biofilms for the water treatment, such as N removal [25]. For instance, Zekker *et al.* reported that biological N removal efficiency in MBBR is dependent on temperature and toxic nitrite concentrations. A high nitrite production (100 mg $NO_2^-$N/L) has an inhibitory effect on ammonium oxidizing bacteria (AOB). Also, they found that AOB populations increased in abundance during reactor operation when the temperature was decreased from 26 °C to 20 °C [26]. In a similar research work, Raudkivi *et al.* reported that nitrite has a limiting effect on Anammox process due to nitrite toxicity [27].

Biofilm processes are commonly used for nitrification [28]. In recent years, many systems have been tested and applied, mainly for the industrial wastewater, to pilot and full-scale plants, such as moving bed biofilm reactors, sequencing biofilm batch reactor, and membrane bioreactors [24, 29]. Different types of nitrifying bioreactors are summarized in Table 1. This review will discuss the most widely used nitrifying bioreactor systems in recent decades which have been proved to be very efficient.

## 2. Biological nitrogen removal

In conventional treatments, the biological removal of nitrogen from wastewater requires a two-step process: autotrophic nitrification and heterotrophic denitrification. During nitrification $NH_4^+$ is converted to $NO_2^-$ and further to $NO_3^-$ with molecular oxygen as the electron acceptor. The oxidation of ammonium is generally attributed to *Nitrosomonas europaea*, and the oxidation of nitrite to *Nitrobacter agilis* [30].

In the second step, denitrification is generally performed by a heterotrophic bioconversion process under anaerobic (anoxic, precisely) conditions. The oxidized nitrogen compounds ($NO_2^-$ and $NO_3^-$) are reduced to gaseous dinitrogen by heterotrophic microorganisms that use nitrite and/or nitrate instead of oxygen as electron acceptors and organic matter carbon and energy source[13]. This process is performed by various chemoorganotrophic, lithoautotrophic, and phototrophic bacteria and some fungi, especially under oxygen-reduced or anoxic conditions [31].

Biological nitrogen removal proceeds slowly because the microorganisms responsible for the removal reactions grow slowly. In addition, the operational control of aerobic and anaerobic conditions needed for nitrification and denitrification, respectively, can be difficult. To cope with these problems, various kinds of bioreactors have been studied for enhancing the efficiency of nitrogen removal [30]. Moreover, generally, the conventional biological nitrogen removal is used for treating wastewaters with relatively low nitrogen concentrations (total nitrogen concentration less than 100 mg N/L) [14]. Several recent studies are beginning to focus on new biological nutrient removal processes, including single reactor system for high ammonia removal over nitrite (SHARON), anaerobic ammonium oxidation (ANAMMOX) [32, 33] and completely autographic nitrogen removal over nitrite (CANON) systems [31].

These new processes are based on the partial nitrification of ammonium into nitrite combined with the anaerobic oxidation of ammonium. Briefly, SHARON process involves the partial conversion of ammonium to nitrite; ANAMMOX presents the anaerobic ammonium oxidation process and CANON process removes nitrogen completely autographic by nitrite in a reactor under limited oxygen conditions. Their most prominent advantages are the reduction of energy demand, the absence of external carbon addition and the lower production of sludge compared to conventional treatment.

Moreover, they offer potential for improving the efficiency of nutrients removal. However, these new technologies meet some challenges for introduction and application in a large-scale plant and need to be optimized for better treatment of contaminated water in high-strength wastewater [31, 34, 35].

## 3. Membrane biofilm reactors

### 3.1. Moving bed biofilm reactor (MBBR)

Moving bed biofilm reactor (MBBR) has been established as a very effective technology for nitrogen removal in wastewater treatment [36]. It has been successfully used for municipal and industrial wastewater including pulp and paper industry wastewater, poultry processing wastewater and dairy wastewater [37-40]. MBBR is being considered as an upgrade option for an increasing number of wastewater treatment facilities due to its small footprint and feasible operation [41]. This process relies on the use of moving carriers in which microorganisms' form biofilms. Thus, the slow-growing microorganisms, such as nitrifying bacteria can be retained in the system without being washed out. However, application of MBBR in wastewater treatment has received much attention now due to their high efficiency [42]. The possible experimental setup of MBBR is shown in Figure 1.

The objective of the MBBR systems is to achieve the growth of the biomass as a biofilm on small carriers, which have a lower density than water [39]. The carriers are continuously kept in the tank and are able to move freely in the reactor without sludge recycling. The systems include a submerged biofilm reactor and a liquid-solid separation unit [43, 44]. Nitrification in moving bed biofilm reactor has been studied to identify the key limiting conditions. In particular, the key factors include the effect of the bulk oxygen concentration, temperature, ammonia concentration and the organic loading rate [45].

According to a study carried out by Wang *et al.*, the nitrification in MBBR depends on DO in the reactor. The DO diffusion through the biofilm was the rate-determining step for media nitrification. The highest nitrogen removal efficiency (89.1%) was reached when the DO was maintained at 2 mg/L. At lower DO concentrations (<1 mg/L), anoxic conditions prevailed and ammonia concentration in the effluent increased [4]. Generally, a high bacterial activity and growing biofilm is the most cause of rapid decrease of oxygen concentration [46].

The MBBR technology promotes biofilm attachment and growth on engineered carriers that are maintained in constant suspension. The attached biofilms are maintained and protected from abrasion with other carriers in the interior spaces of the MBBR carriers [15]. Nitrifying biofilms attached to MBBR carriers have demonstrated highly diverse microbial populations [47]. Several parameters may affect the efficiency of the MBBR such as the percent of media provided in the reactor and the organic loading (to allow the free carrier suspension, the percentage of reactor volume occupied with carriers in empty tank normally varies from 60% to 70%) [48]. Young *et al.* stated that the MBBR performance depends on the loading rate as well as the carrier type. The carriers play an important role in the formation of microbial community within the biofilm. According to their observations, the pore spaces of carriers with the higher surface area to volume (500 $m^2/m^3$ to 900 $m^2/m^3$) has potential to become clogged at severe conditions in synthetic wastewater [49]. Different types of carriers have been used in MBBR systems such as polyethylene plastic, polyurethane sponge, granular activated carbon, etc. [50, 51]. Martín-Pascual *et al.* used commercial carriers (Aqwise, Kaldnes, and BIOCONS) in MBBRs for treatment of municipal wastewater and obtained chemical oxygen demand (COD) removal of 56%, 58 % and 46%, respectively [52]. Among the carriers, sponge exhibited an ideal attached growth media due to its high porosity and immobilization tendency of biomass on the surface and inside of the sponge pores [53, 54]. Zhang *et al.* used sponge cube with a specific surface area of 0.846 $m^2/g$ for treatment of synthetic wastewater in MBBR [53]. Similarly, Nguyen *et al.* studied the organic removal in a bioreactor with sponge cube as a carrier. They obtained over 90% removal in total organic carbon (TOC), 95% removal in COD, 90% removal in total phosphorus and 65% removal in total nitrogen aerobic conditions [55]. Deng *et al.* used a sponge modified carriers with a specific surface area of 500 $m^2/m^3$ for treatment of domestic wastewater and observed an improvement in the effluent quality and nutrient removal, compared to the MBBR using plastic carriers [54]. Chen *et al.* coated the inside and outside of a hard polyethylene ring with a sponge to use as a carrier in MBBR. COD and ammonium removal reached 99% and 93%, compared to commercial carrier (74% and 40.0%) [56]. Chu *et al* used a biodegradable polymer as biofilm carriers in a MBBR for simultaneous nitrification and denitrification in wastewater. They obtained 74.6% total nitrogen removal efficiency on average [57]. They also studied the performance of two types of carriers namely polyurethane foam and polycaprolactone on the removal of nitrogen and organics from wastewater. The polyurethane foam exhibited higher removal for TOC and ammonium (90 and 65% versus 72% and 56%, respectively) [58].

The biofilm growth can be affected by the constant collision and shear of media, the effective internal surface area is an important design factor [36]. Trapani *et al*. reported that the fill fraction is an important parameter which must be considered depending on the treatment objectives. They concluded that fill fraction is responsible for the creation of competition between suspended and attached biomass and the importance of suspended solids which can decrease the MBBR efficiency. However, low suspended biomass can decrease the MBBR nitrogen removal efficiency due to their major role in enzymatic hydrolysis and bio-flocculation in the reactor [59]. Hem *et al.* reported that nitrification rates using biofilm reactors are straightly influenced by the DO concentration, total ammonium nitrogen (TAN) concentration, organic load, temperature and pH [45, 60].

As shown by Monfet et al., anaerobic AOB process has been widely used for nutrient recovery and it has recently considered as a novel route for N removal in MBBR systems[61]. Szatkowska *et al*. studied a simultaneous partial nitritation/Anammox process in MBBR for completely autotrophic nitrogen removal. They reported that bacterial culture was able to perform simultaneously two processes, Anammox and partial nitritation in a single-stage reactor at the technical-scale pilot plant. The maximum nitrogen removal rate (1.45g N $m^{-2}$ $d^{-1}$ ) was obtained for the pilot plant during the 1-year experimental period [62]. In another study by the same authors, it has been proven that biofilm entrained Anammox in MBBR will survive at temperatures below the range 30 °C to 35 °C [63].

Finally, MBBR has been recognized as an ideal process for nitrification, it allows a good bacterial proliferation due to the carriers while allowing to sustain a high-density population of nitrifying bacteria [36].

**3.2. Membrane aerated biofilm reactor (MABR)**

Membrane aerated biofilm reactors (MABR) are commonly understood as the combination of membrane filtration and biological treatment using activated sludge (AS) where the membrane primarily serves to replace the clarifier in the wastewater treatment system [64]. The MABR is an emerging technology for wastewater treatment. It is based on pressurized membranes that supply a gaseous substrate to a biofilm formed on the membrane exterior. MABR behaves differently from conventional biofilms due to the counter-diffusion of substrates. They are uniquely suited for numerous treatment applications, including the nitrogen removal when oxygen is supplied [23]. Martin *et al.* observed that complete nitrogen removal (100%) can be achieved by membrane biofilm reactors [23]. Figure 2 shows a schematic diagram of the laboratory-scale MABR that is used for nitrification.

Recently, there has been a growing interest in reactor systems using MABRs because of their several advantages over conventional systems, their ability to remove nitrogen and for their efficient potential low energy oxygen transfer [65]. MABR represent a new technology for aerobic wastewater treatment. Oxygen diffuses through a gas permeable membrane into the biofilm where oxidation of pollutants, supplied on the biofilm side of the membrane, takes place [66]. In the MABR, the biofilm grows on an oxygen-permeable membrane. It allows a simultaneous nitrogen and organics removal [67]. Hibiya *et al.* reported 90% removal of total nitrogen from modified domestic wastewater up to 150 days by using the MABR [67]. COD and nitrogen removal rates obtained from published MABR trials are consistently higher than any other wastewater treatment technologies processes in current use, such as MBBRs [68]. Figure 3 shows schematically the diffusive and advective fluxes of oxygen and soluble wastewater constituents in an MABR.

In addition, MABRs attain higher gas transfer rates compared to conventional bubble aeration. The simultaneous removal of COD and nitrogen from wastewater is ensured by MABR with smaller tank sizes with significant energy savings and specific microbial communities [69]. Wang *et al.* reported that the ammonium removal efficiency of the MABR was increased from 50% to 90% by increasing the transmembrane pressures from 0.002 to 0.02 MPa, [69]. Tian *et al.* operated an MABR at several aeration pressures (0.1, 0.15, and 0.2 MPa) with a same hydraulic retention time (HRT) (24 h) for the treatment of wastewater. By increasing the aeration pressure, COD and ammonium concentrations of effluent decreased, and reached to the lowest values of around 75 mg/L and 0.4 mg/L, for aeration pressure of 0.2 MPa. Higher air supply pressure could be in favor of nitrification rate, but might be limiting the denitrification process to a certain degree [70]. The aeration pressure also play an important role in microbial stratification and can affect nitrification and denitrification [71, 72]. Li *et al.* demonstrated that increasing aeration pressure from 0.005 to 0.03 MPa, led to increase in the COD/N ratio from 4 to 7 [73]. Similarly, Syron *et al.* observed that when oxygen pressure was increased from 0.015 to 0.02 MPa, complete oxidation to nitrate happened and oxygen transfer efficiency was increased from 20% to 80% [74].

The simultaneous nitrification and denitrification are allowed when the oxygen delivery through the membrane is precisely controlled [67]. According to a study carried out by Yamamoto *et al.* a handling trans-membrane air pressure provision can control the nitrification rates in MABR. They also reported that the ratio of the oxygen flux to the ammonia flux was the crucial parameter for controlling nitrogen conversion [75]. Lackner *et al.* demonstrated that the oxygen transfer rate and partial pressure can determine the success of nitrification in MABRs. Furthermore, high oxygen concentration at the biofilm base compromised the ability to optimize reactor operation for high nitrification efficiency by adjusting the oxygen flux via the gas pressure [76]. Very high specific nitrification rates with close to 100% oxygen conversion efficiency in a sealed end hollow fiber MABR was reported by Brindle and Stephenson [77, 78]. Due to thin biofilms and a high bulk liquid DO concentration, it is likely that complete oxygen penetration of the biofilm occurred which resulted in specific nitrification rates of 13 kg $NH_4$-N kg/(SS day), significantly higher than most other nitrification processes. If the biofilm thickness can be kept low, then the volumetric nitrification rate is only limited by the specific membrane surface area available for the biofilm attachment [77, 78]. Downing and Nerenberg found that the ammonium flux and nitrite accumulation increased with increasing transmembrane operation pressure and that shortcut nitrification can effectively be controlled by varying DO at the membrane surface [79]. One of the applications of MABRs is to control the rate of nitrification and, if possible, create conditions promoting simultaneous nitrification and denitrification [4]. One of the claimed advantages of MABRs is that they can be used for simultaneous nitrification and organic removal in a single reactor. Low rates of nitrification but very high organic carbon oxidation were reported by several researchers in studies where a washing procedure was employed to detach excess biomass [80, 81]. MABR studies by Miyahara *et al.* showed that the thickness of a denitrifying layer affected the oxygen transfer rate and that it was necessary to control the biofilm thickness by sloughing in order to maintain oxygen transfer rates high enough for effective nitrification [82].

### 3.3. Biofilm airlift suspension reactor (BASR)

The biofilm airlift suspension reactor (BASR) is an attached growth system that consists of two concentric tubes. Air is introduced at the bottom of the inner tube of the airlift part. A schematic representation of the BASR is given in Figure 4.

The introduction of air creates a difference between the fluid density in the riser (rising column) and that in the downcomer. As a consequence, it creates mixing between the liquid and the carrier. In addition, air added to the reactor provides the oxygen needed to degrade the substrate. On top of the airlift part, there is a three-phase separator which is used to retain the biofilm particles in the reactor [83]. Airlift technology reactor represents a potential solution where the high oxygen levels in the stream assure higher efficiency and low odor impact. However, the design and management of these less common treatment plants can require a numerical tool to analyze and control the different processes involved [84]. Several mathematical models were developed to link the substrate flux into the biofilm to the fundamental mechanisms of substrate utilization and mass transport. Nitrification can be performed efficiently in BASR [85].

In biofilm systems, the maximum volumetric ammonia conversion is usually limited by the liquid-biofilm or the gas-liquid oxygen mass transfer rate. BASRs have a relatively high gas-liquid mass transfer of oxygen and a high specific area due to the growth of a biofilm on small suspended carrier particles. This makes it possible to reach high volumetric ammonia conversions [2, 21]. The biofilm airlift suspension reactor is well suited for nitrification. This compacted reactor combines a high nitrification capacity and a high biomass concentration with a low ground area occupied because the biomass settler is integrated on top of the reactor [21]. The inherent benefits of the BASR are attributed to its high oxygen transfer efficiency, a high concentration of immobilized biomass on the solid carrier, and excellent mixing and substrate transfer abilities [83]. Experimental observations of Garrido *et al.* have shown that it is possible to obtain full ammonium conversion with approximately 50% nitrate and 50% nitrite in the effluent of a biofilm airlift suspension reactor. With oxygen concentrations between 1 mg/L and 2 mg/L, a maximum nitrite accumulation of 50% was reached [86]. According to Picioreanu *et al.* controlling the oxygen concentration seems to be the most practical method to obtain optimal nitrification in BAS reactors. They concluded that varying the oxygen concentration was the most practical method to obtain partial nitrification in BASR since this can be done by varying the superficial gas velocity or by partial recirculation of the off-gas [87]. In a study done by Van Benthum *et al.*, they concluded that in BASR, it is possible to have process and oxygen control to force the nitrification, thereby saving needed COD [20].

### 3.4. Sequencing batch biofilm reactor (SBBR)

The sequencing batch biofilm reactor (SBBR) system is a biofilm technology which has attracted much attention because of its ability to take advantage of being both a biofilm reactor and a sequencing batch reactor [88]. The SBBR system shows higher biomass concentration in the reactor, with corresponding higher specific removal rates and less sludge production, higher volumetric loads, increased process stability towards shock loadings and biomass enrichment of slow-growing organisms, such as nitrifiers than the competing technologies [89, 90]. The SBBR is a fill and draw reactor where the biomass is fixed on a support medium. In this system, wastewater is added to a single batch reactor and treated to remove undesirable components before discharge [89]. As compared to most activated sludge sequencing batch reactor (SBR) systems, which require the settling period to separate activated sludge, the SBBR system typically does not need settling and sludge recycling equipment, still maintaining high microbial concentrations inside the reactor [91]. The schematic of sequencing batch reactor mechanism is shown in Figure 5.

Accordingly, the SBBR has been adopted to remove nitrogen and phosphorus simultaneously from many types of wastewater [92]. Jin *et al.* reported that the total nitrogen removal was significantly influenced by nitrogen loading rate and better nitrogen removal was achieved at higher C/N ratios. The average of nitrogen removal efficiencies was varied between 65.4% to 81.0% [92]. Many successful cases of partial nitrification have been reported for SBBR. There are few reports about partial nitrification conducted in an intermittently aerated SBBR [93]. SBBRs are spatially heterogeneous, providing space for both, aerobic and anaerobic processes. They are well suited for nitrification since the attached growth of the slow-growing nitrifying bacteria protected them from washout [94]. Recently, an SBBR was designed for efficient enhanced biological phosphorus and nitrogen removal and is successfully scaled up to the pilot scale [95]. The implementation of nitrification into this type of reactor is a challenging but desired step in saving reactor volume and costs. As for nitrification and phosphorus removal, both processes consume oxygen, hence the organisms in such a system are potentially subjected to competition for oxygen [96].

Nitrification and nitrifying bacteria were always restricted to the periodically oxic biofilm surface. Both activity and population size increased significantly with higher ammonium concentrations. Nitrification always showed a delay after the onset of aeration, most likely due to competition for oxygen by the co-existing phosphorus accumulating and other heterotrophic bacteria during the initial aeration phase. This view is also supported by comparing oxygen penetration and oxygen uptake rates under low and high ammonium conditions. Therefore, simultaneous nitrification and phosphorus removal in a phosphorus removing SBBR appear to be only possible with a sufficiently longer oxic period to ensure oxygen availability for nitrifiers [97]. Malaspina *et al.* found that SBBR showed high N-removal capacity with excellent sludge settling properties. On the other hand, organic carbon removal efficiency with nitrate was lower than with oxygen. Also, batch biofilm nitrification was very effective, with very high nitrification rates [98].

In conventional biofilm reactors, autotrophic nitrifying organisms may be excluded from the oxic layer of the biofilm due to the faster growth of heterotrophs. As a result, substantial nitrification only occurs when the carbon substrate loading rate of the wastewater is low [66]. Zuniga and Martinez reported an efficient phosphate removal and complete nitrification using SBBR [99]. According to Wei *et al.*, partial nitrification was successfully achieved and maintained in an SBBR [100]. Although fixed-bed reactors have been shown to be capable of treating a variety of wastewaters, the real-world applications of SBBRs are limited due to several drawbacks of the system itself. For example, the fixed bed is easily clogged [101]. Recently, it was suggested that moving bed biofilm reactors (MBBRs) could be operated in a sequencing batch mode, in order to benefit from the advantages of both processes (Table 2) [102]. The use of moving bed sequencing batch reactor (MBSBR) which combines suspended growth and attached growth processes in a single reactor to remediate nitrogen bearing wastewater has gained increasing interest among the researchers [103]. Tan *et al.* studied the performance of total nitrogen removal by MBSBR and removal efficiency was in the range of 64% to 80%. He reported that influent feed has a positive effect on nitrogen removal [35, 103].

## 4. Operating conditions in biofilm reactors

The influence of operational parameters on the nitrification of the biofilm is illustrated in Table 3. Factors, such as pH, DO, HRT and temperature can affect the TN, COD and total phosphor removal. Pandey and Sarkar observed that by increasing HRT from 18 h to 72 h, the COD removal efficiency increased from 61% to 89% [104]. Kim *et al.* observed that by increasing HRT from 3 h to 4 h, nitrification efficiency was increased from 75% to 82% [105]. Brosseau *et al.* studied the performance of bioreactors at 10 °C and 20 °C and reported that the decreasing the temperature from 20 °C to 10 °C caused a significant reduction of 15-41% of COD removal efficiency [106]. Li *et al.* reported that supplying sufficient DO (5 mg/L) and avoiding the negative effect of the aeration shear stress help to form a biofilm with enough thickness [107].

The polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) is a method for identifying the most abundant bacteria in the bioreactors system [108]. Conventional PCR can provide information on the presence of certain microorganisms but does not provide any information on abundance. Recently, quantitative real-time PCR (qPCR) was developed to provide quantitative information on the abundance of a certain microorganism using fluorescently labeled probes and dyes [109]. Pellicer-Nacher *et al.* studied the abundance of functional microbial guilds after 630 days of MABR operation via qPCR techniques. Their results revealed 0.2%, 5.4% and 25% relative abundance for NOB, AOB and anaerobic ammonium oxidizing bacteria, respectively [110]. Torresi *et al.* studied the effect of thickness of biofilm on the microbial community through qPCR technique. Their results showed that the biofilm with the highest thickness (500 μm) achieved the highest constants for specific biotransformation rate and the biofilm with least thickness (50 μm) showed the highest nitrification rate [111].

**5. Cost factors**

Operational cost savings are necessary for the membrane to be competitive. It can be reached through reduction of energy consumption, the elimination of brine release, employing low-cost carrier, etc. [54, 112]. Nerenberg *et al.* reported that a major advantage of the membrane biofilm reactor is low energy requirement (86% more energy efficient than conventional system). Membrane durability, membrane cost, and membrane removal fluxes are other factors affecting the cost saving. Without full-scale data, it is difficult to quantify the savings, but data obtained from the pilot scale and modeling can be used as a primary approximation. [112, 113]. In an MABR, aeration is very energy intensive and accounts for 45-75% of plant energy costs [114]. However, MABR can outperform conventional treatment in terms of energy efficiency and pollutant removal rate due to high oxygen transfer efficiencies and smaller aeration equipment. Operating MABR with pure oxygen requires up to five times less membrane area compared to operating with air which provides savings in capital investment [74]. Also, Lin *et al.* emphasized on the cost-effectiveness of MABR compared to conventional biological treatment because of lower emission of volatile pollutants and lower operating cost. The effectiveness of membranes in MABR is twofold compared to conventional bubbled diffuser reactors, due to immobilizing microorganisms on carriers and less required aeration [115]. Hem *et al.* reported the higher sensitivity of nitrification in the MBBR to the variation of oxygen concentration compared to other biofilm reactors. Due to this sensitivity, the nitrifying moving bed reactor is generally operated at a higher oxygen concentration to reduce the reactor size and consequently, the construction costs [45, 54]. Cost of membrane replacement is the main economic obstacle to commercial scales. However, the decreasing trend observed in the cost of the commercial membrane in recent years and also increase in the cost of energy is promising to make the membrane bioreactors more attractive. In conclusion, more laboratory scale and recent pilot scale data are required to generate real-world data for economic evaluation.

**Conclusion and Future work**

The conventional process for nitrogen removal from wastewater comprised of autotrophic nitrification and heterotrophic denitrification. Due to high consumption of energy and carbon source, more research is needed for development and implementation of economically efficient processes. The different biofilm reactors, such as moving bed reactor, membrane aerated biofilm reactor, biofilm airlift suspension reactor and sequencing batch reactor are widely used for a number of wastewater treatment applications. To date, they are particularly suitable due to their high-rate nitrification and nitrogen removal in the laboratory and large-scale demonstrations. There are a large number of reports comparing the performance of biofilm reactors as a promising technology to achieve high efficiency in nutrient removal. Briefly, the sequencing batch biofilm reactor is a very useful system due to its low cost and the flexibility of its operation. The moving bed biofilm reactor is also an equally efficient system since it promotes the development of biomass and does not produce a large amount of sludge compared to other systems. The membrane aerated biofilm reactor provides an optimal concentration of oxygen to the biomass. And finally, the biofilm airlift suspension reactor is a system that allows a high mass and oxygen transfer rate and it has a high nitrification capacity.

Recent investigations on the modeling and engineering aspects of different biofilm reactors indicated the significance ($P < 0.05$) of the placement of individual microbial layers in biofilms. Understanding the processes affecting the placement of these individual layers are required for reactors' scale-up and also for the selection of the optimum conditions.

Despite the commercial implementation of biofilm reactors for wastewater treatment and production of value-added products, the knowledge of fundamentals of biofilm formation and physicochemical properties of a biofilm is required to run the reactor at optimum conditions. Moreover, the stable productivity of bioreactor can only be achieved through optimum reactor design and by improving solid supports for homogeneous distribution of the biofilm. In addition to the above-mentioned significant factors, other parameters, such as wastewater characteristics, biofilm composition/structure, and carrier-biofilm interaction must be considered for each product and microorganism to skip the restrictions imposed by diffusion, biomass activity, etc.

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**Table 1:** Overview of cultures used in different types of nitrifying bioreactors with P-value (< 0.05)

| Cultures | Condition | Biomass | Removal | Removal comparison | Reactor type | Reference |
|---|---|---|---|---|---|---|
| Anaerobic sludge | pH: 7.5<br>Temperature: 30 °C | - | 50% (N-$NO_3^-$) | Medium | Batch system | 18 |
| Wastewater | pH: 7.6<br>Volume: 2.5 L<br>Temperature: 30 °C | 0.06 (kg/kg) | 83% (N-$NO_3^-$)<br>98% (N-$NH_4^+$) | High<br>Very high | MABR[a] | 78 |
| Synthetic wastewater | pH: 7.6<br>Volume: 3 L<br>Temperature: 30 °C | 17-70 g/L | 50% (N-$NO_2^-$)<br>50% (N-$NO_3^-$)<br>100% (N-$NH_4^+$) | Medium<br>Medium<br>Very high | BASR[b] | 116 |
| Wastewater | pH: 7.8-8.5<br>Volume: 50 L<br>Temperature: 18.6-27.6 °C | - | 61% (COD[c])<br>62% (N-$NH_4^+$) | Medium<br>Medium | BAF[d] | 25 |
| Wastewater | pH: 7.5<br>Volume: 10 L<br>Temperature: 18.6-27.6 °C | - | 60% (COD)<br>95% (N-$NH_4^+$) | Medium<br>High | SBMBBR[e] | 117 |
| Secondary municipal effluent | pH: 7.6<br>Temperature: 25 °C | - | 99% (N-$NH_4^+$) | Very high | MBR[f] | 77 |
| Synthetic wastewater | pH: 7.3<br>Temperature: 30 °C | 276 and 562.5 (mg/g) | 97% (N-$NH_4^+$) | Very high | SBBR[g] | 118 |
| Synthetic wastewater | pH: 8<br>Volume: 1.2-2 L<br>Temperature: 22 °C | - | 99% (N-$NH_4^+$) | Very high | MBBR[h] | 41 |
| Domestic wastewater | pH: 7-8<br>Volume: 5 L<br>Temperature: 25 °C | - | 17-90% (N-$NH_3$) | Medium to high | MBBR | 3 |
| Activated sludge | pH: 7<br>Volume: 3 L<br>Temperature: 20 °C | 2.5-10 (g/L) | 50% (COD) | Medium | SBAR | 119 |
| Municipal wastewater | - | - | 89% (COD)<br>87% (N-$NH_4^+$) | High<br>High | SBBR | 99 |
| Domestic wastewater | Volume: 100 mL<br>Temperature: 30 °C | - | 89% (TOC)<br>89% (T-N) | High<br>High | MABR | 67 |
| Wastewater | pH: 7.85-8.15<br>Volume: 371 L<br>Temperature: 29.36- 35.70 °C | - | 69.2% (COD)<br>95% (N-$NH_4^+$) | Medium<br>Very high | MBBR | 42 |
| Wastewater | pH: 7-8<br>Volume: 50 mL<br>Temperature: 15-35 °C | - | 91% (N-$NH_4^+$) | High | SBBR | 91 |
| Synthetic waste water | pH: 6.8-7<br>Volume 1.1L<br>Temperature: 35 °C | 42.0-57.7 (g-VSS/L) | 74.3–76.7 (kg-N $m^{-3}$ $day^{-1}$) | High | UASB[i] | 120 |
| Domestic waste water | pH: 6.5-7<br>Volume 20 L<br>Temperature: 5-15 °C | - | 26-32% ($NH_4^+$-N)<br>71-75% (COD)<br>85-88% (SS)<br>71-75% ($PO_4^{3-}$-P) | Medium | Septic tank | 121 |
| a: Membrane aerated biofilm reactor, b: Biofilm airlift suspension reactor, c: Chemical oxygen demand, d: biological | | | | | | |

aerated filter, e: sequencing batch moving bed biofilm reactor, f: membrane bioreactors, g: Sequencing batch biofilm reactor, h: Moving bed biofilm reactor, i: upflow anaerobic sludge bed reactor

**Table 2:** Advantages and disadvantages of nitrifying systems

| System | Advantages | Disadvantages | References |
|---|---|---|---|
| SBBR | Operating flexibility and control<br>Potential capital cost savings<br>Nitrogen and phosphorus removal<br>Reduce the requirement for substrates<br>High biomass retention and concentration<br>Elimination of long sludge settling periods<br>Minimal footprint | Limited real-world applications<br>Lots of sludge amount<br>High sludge volume index<br>A higher level of maintenance<br>Potential of discharging floating or settled sludge | 107, 122-125 |
| MBBR | Compact units with small size<br>Increased treatment capacity<br>Complete solids removal<br>Improved settling characteristics<br>Operation at higher suspended biomass<br>Concentrations (long sludge retention times)<br>Enhanced process stability<br>Low head loss<br>No filter channeling<br>No need of periodic backwashing<br>Reduced sludge production<br>No sludge bulking | Energy Consumption<br>Coarse Bubble<br>Higher DO<br>Influent Screening<br>Tank Downtime<br>Media Procurement<br>Scum, Foam<br>Restriction of biofilm growth | 44, 126-129 |
| MABR | Simultaneous nitrification and denitrification<br>High volumetric carbon oxygen demand<br>High resistance to shock loadings<br>Higher oxygen utilization<br>Low solids production<br>Low maintenance | Difficulty in maintaining an optimum biofilm thickness<br>High costs of liquid pumping<br>Difficulty in scale-up | 68, 130-132 |
| BASR | Faster mass and oxygen transfer rate<br>High ammonia conversion to nitrate<br>High nitrification capacity<br>High biomass concentration | High sludge production | 2, 116, 119 |

**Table 3:** Effect of different operation condition and their related result with P-value ($< 0.05$)

| DO concentration | HRT | pH | Temperature | COD removal | TN removal | TP removal | Reference |
|---|---|---|---|---|---|---|---|
| mg/L | h | - | °C | % | % | % | - |
| 4 | 12 | 6.5-7.5 | | 86 | 26 | 0 | [133] |
| 5 | 10 | 6.5-7.5 | 25 | 98 | - | - | [134] |
| - | 8 | 7 | 25-28 | 82.2-95.6 | 97-100 | - | [135] |
| 1-4 | 12 | 4.5-7 | 20 | 81.6 | 75.8 | - | [136] |
| 5.5 | 12 | - | 30 | 96.3 | 84.9 | 93.9 | [137] |
| 3-3.5 | - | 7.9-8.3 | 25-35 | 72 | 31 | - | [138] |
| 2.5-3.5 | - | 7.6–7.8 | 20 | 93 | - | - | [139] |
| 3.0-4.0 | 11.9 | 7.3-7.5 | 30-32 | 85 | 83 | - | |

DO: Dissolved Oxygen; HRT: Hydraulic Residence Time; COD: Chemical Oxygen Demand; TN: Total Nitrogen; TP: Total Phosphorus

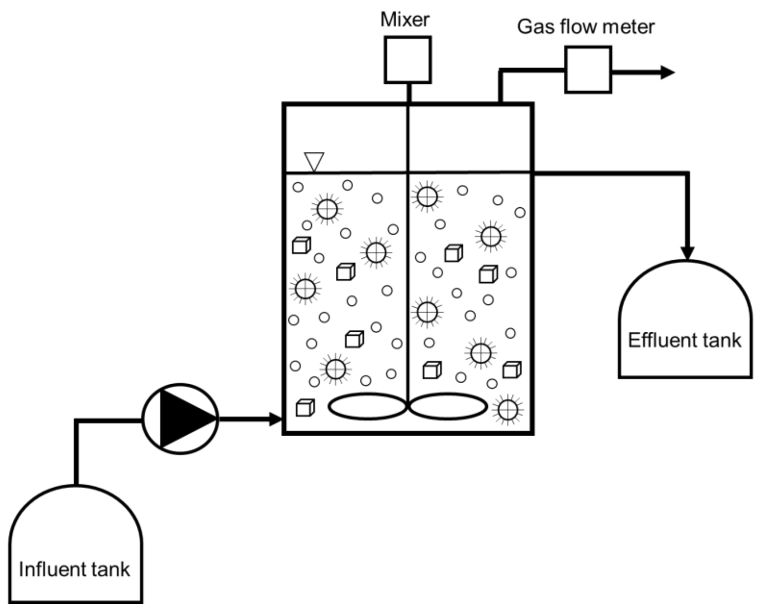


**Figure 1:** Schematic diagram of the moving bed biofilm reactor

The moving bed biofilm reactor may have one or more stages. The bacteria stay in the duty tank because the carriers are protected by screens. The bacteria grow on the surface of the carriers and break down the organic materials in wastewater. The carriers are kept in motion by an aeration system. The excess bacteria will be separated from the carriers and will flow with the effluent to the final separator [140].

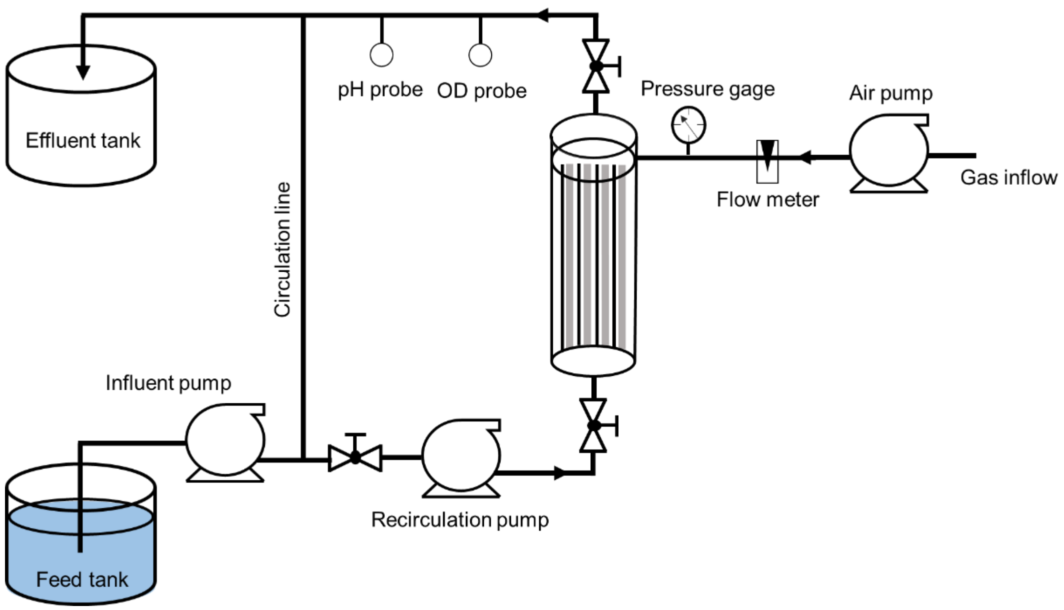


**Figure 2:** Schematic diagram of the membrane-aerated biofilm reactor

In the membrane-aerated biofilm reactor, membranes are immersed vertically into the reactors. First, the reactor is filled with distilled water and nitrogen is sparged into the reactor in order to decrease dissolved oxygen. Then, aeration is started through the hollow-fiber membrane [75].

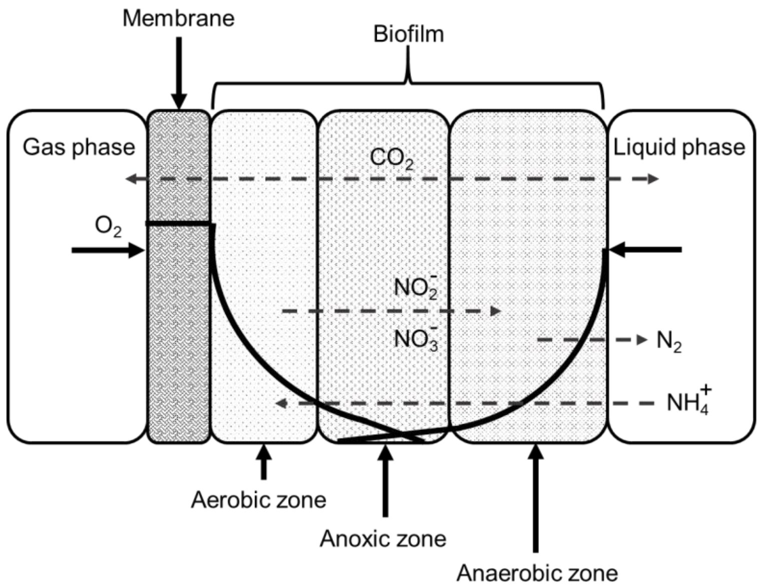


**Figure 3:** Schematic of oxygen profile in membrane-aerated biofilm

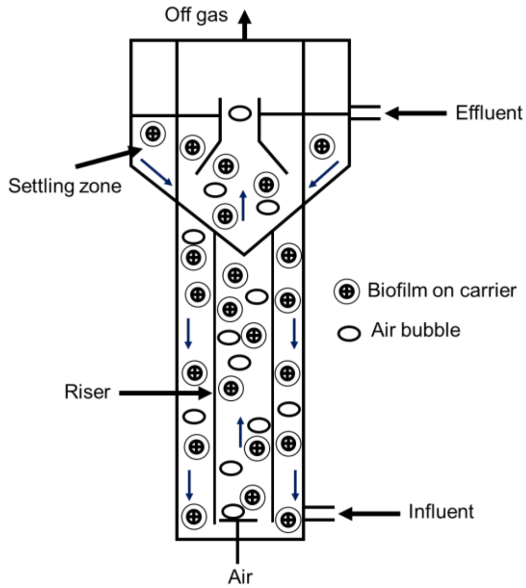


**Figure 4:** Schematic diagram of the biofilm airlift suspension reactor

The biofilm airlift suspension reactor has three parts: riser, down-comer, and three-phase separator. Inertial materials, such as activated carbon are used as carriers to for growth of microorganisms. Upon initial aeration through the riser, the density difference between the riser and down-comer, results in the internal circulation of carriers, wastewater, and bubbles. Carriers are uniformly distributed through the reactor and bacteria have close contact with wastewater to degrade organic compounds [141].

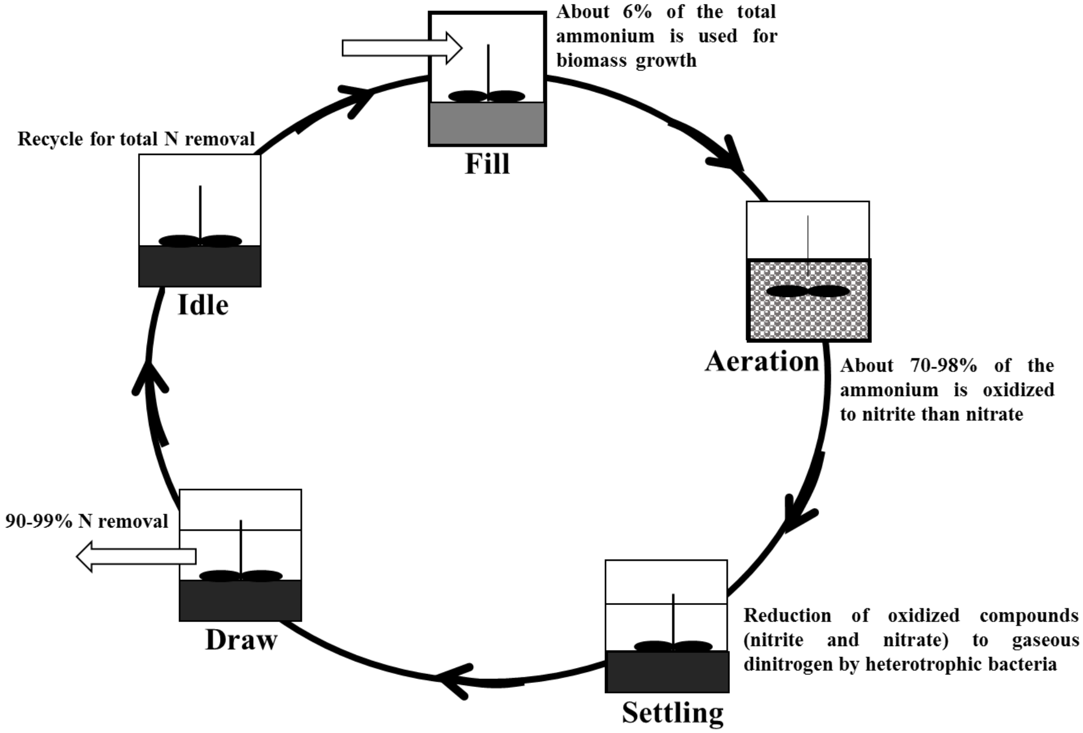


**Figure 5:** Schematic of sequencing batch reactor mechanism