Review Paper:

Existing biological nitrogen removal processes and current scope of advancement

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Abstract
In India, to achieve the stringent norms of total nitrogen less than 10 mg/l in sewage treatment plant is a big challenge for the public - private facilities and organizations. After successful implementation of this norm the pollutant burden from rivers and natural water bodies certainly reduces. The use of conventional biological nitrogen removal (BNR) processes for new treatment facility development or retrofitting is also an energy and cost intensive practice. The process technologies offered by current market such as MLE, MBBR, IFAS and SBR are still in with downside of higher footprint, multi tank reactors, heavy instrumentation for IR and RAS which ultimately incur higher capital and operating cost. The current market need and lack of sustainable nitrogen removal applications, trigger to review the of all available efficient biological nitrogen removal processes. This review will gives an overall scenario of past and current biological nitrogen removal process technologies with showing possible scope and way forward towards more energy neutral nitrogen removal technologies.

Keywords: Nitrogen removal, sewage, footprint, energy, sustainable

Introduction

Underground water is already overexploited in the United States, India and China **a**nd providing the water needed to feed a growing population and balancing this with all the other demands on water, is one of the great challenges of this century[38]. People living in river basins under severe water stress are projected to more than double between 2000-2050, reaching 3.9 billion[39]. Almost one fifth of the world's population lacks the access to safe drinking water and this water crisis is leading to cause of death and disease in the world, with more than 14000 people dying each day[38]. Currently pressure for water is growing on natural systems and urgent steps must be taken to begin to implement tried and tested methods of wastewater treatment before the situation gets out of control. With the organic pollutants, fixed nitrogen such as ammonium and nitrate must be removed to avoid the eutrophication in water bodies.

Lower NH_3-N concentration at 1.68 mg/l also showed its toxic effect on fish flora[52]. Ammonia also imparts an oxygen demand in natural water systems because nitrifying bacteria will consume dissolved oxygen (DO) while oxidizing ammonia to nitrite and nitrate. Nitrate levels above 10 ppm may present a serious health concern for infants and pregnant or nursing women[20].

Also, the current scenario of wastewater treatment is facing towards the development and use of energy efficient technologies. Method of pumping air into the wastewater is defined as aeration process and it takes 55.6% of total energy consumption in the wastewater treatment[41]. Further advanced treatment with nitrification required 40% more energy than conventional one[15]. In case of total nutrient removal, anoxic recycling for denitrification with anoxic or anaerobic mixing also increases the energy consumption. In recent times, the novel biological nitrogen removal (BNR) mechanisms were identified such as Simultaneous Nitri-Denitrification (SND)[25], Nitritation-Denitritation[49] and Nitritation-Anaerobic Ammonium Oxidation (ANAMMOX)[32] methods. These methods offer the economically attractive and environmentally friendly alternative to current wastewater treatment processes for the removal of fixed nitrogen[29].

Current high rate wastewater treatment technologies include the moving bed biofilm reactor (MBBR) and integrated fixed film activated sludge (IFAS) process. The MBBR is known for completely mixed, continuously operated, compact and its simplicity of operation[7,9,11] with major benefits of lower footprint, high degradation/reaction rate, nitrifiers retention and its flexibility of operations at varying load. There are also some process disadvantages of MBBR such as high operating cost, chemical requirement, settlers for sludge separation and high sludge production. These energies and cost intensive limitations need to be minimized, to accept MBBR as an ideal process. The MBBR used for high rate COD or BOD removal applications and second reactor can be attached for nitrification whereas the IFAS process can be used for total nitrogen removal by using high hydraulic retention time (HRT) and as efficient nitrogen removal processes with a sludge recycling cost[65].

The increasing demands of water and energy with increase of population will be unavoidable whereas the energy resources remain same. This paper reviews the current biological nitrogen removal processes used in wastewater treatment and scope for further sustainable development in nitrogen removal process.

Existing Processes of Biological Nitrogen Removal Conventional wastewater treatment systems for nitrogen removal require a lot of energy to create aerobic conditions for bacterial nitrification with use of organic carbon to remove nitrate by bacterial denitrification[30]. Major biological nitrogen removal process reactions are as follows:

Nitrification – Denitrification: Conventional nitrogen removal process follows the nitrification- denitrification pathway (Figure 1a) where the autotrophic two step nitrification is carried by *Nitrosomonas sp.* and *Nitrobactr sp.*

Aerobic Denitrification: Simultaneous nitrification and denitrification (SND) process occur concurrently in the same aerobic reactor inside of floc structure (Figure 2) which remove 80 to 96 % nitrogen without additional carbon and alkalinity requirement. The C:N ratio is required to be 10 and the bulk dissolved oxygen (DO) concentrations need to be maintained is between 0.3 mg/l to 0.7 mg/l[25]. The reaction depends on DO level, sludge size and diffusion barriers[24].

Nitritation – Denitritation: The nitritation- denitritation process is also called as “Nitrite Shunt” which avoids the oxidation of nitrite to nitrate by nitrite oxidizing bacteria (NOB) and allows for the reduction of the formed nitrite to dinitrogen gas by heterotrophic denitrification (Figure 1b). It would decrease the organic carbon demand for total nitrogen removal by 40%. Additionally, 25% of the aeration costs can be saved by avoiding nitrite oxidation[62,70]. It is reported that combination of controlled aeration phase length and DO at 15°C resulted in nitritation- denitritation reactions under aerobic granular sludge in SBR, resulted in total nitrogen removal efficiency of up to 95%[33]. Similar studies on nitritation – denitritation were carried out in SBR form of system with maximum 96% nitrogen removal with ammonia-rich landfill leachates[13].

Nitritation – ANAMMOX: Application of anaerobic ammonium oxidizing (ANAMMOX) process for the treatment of high nitrogen strength wastewater is an emerging technology and scope for lower oxygen requirement. In the process (Figure 1c), aerobic ammonia oxidizing bacteria (AOB) oxidize half of the ammonia to nitrite which is used by ANAMMOX bacteria (*Candidatus Brocadia fulgida*) as electron acceptor for oxidizing the remaining ammonia to dinitrogen gas[2].

The quantitative analysis of oxygen consumption in a partial nitrification-ANAMMOX biofilm process[16] and effect of granule size on autotrophic nitrogen removal[66] was studied in the ANAMMOX process. In 2014, the group of Delft University also reported the simultaneous partial nitritation and ANAMMOX process[35] with physiological and kinetic characterization of a suspended cell ANAMMOX reaction[34].

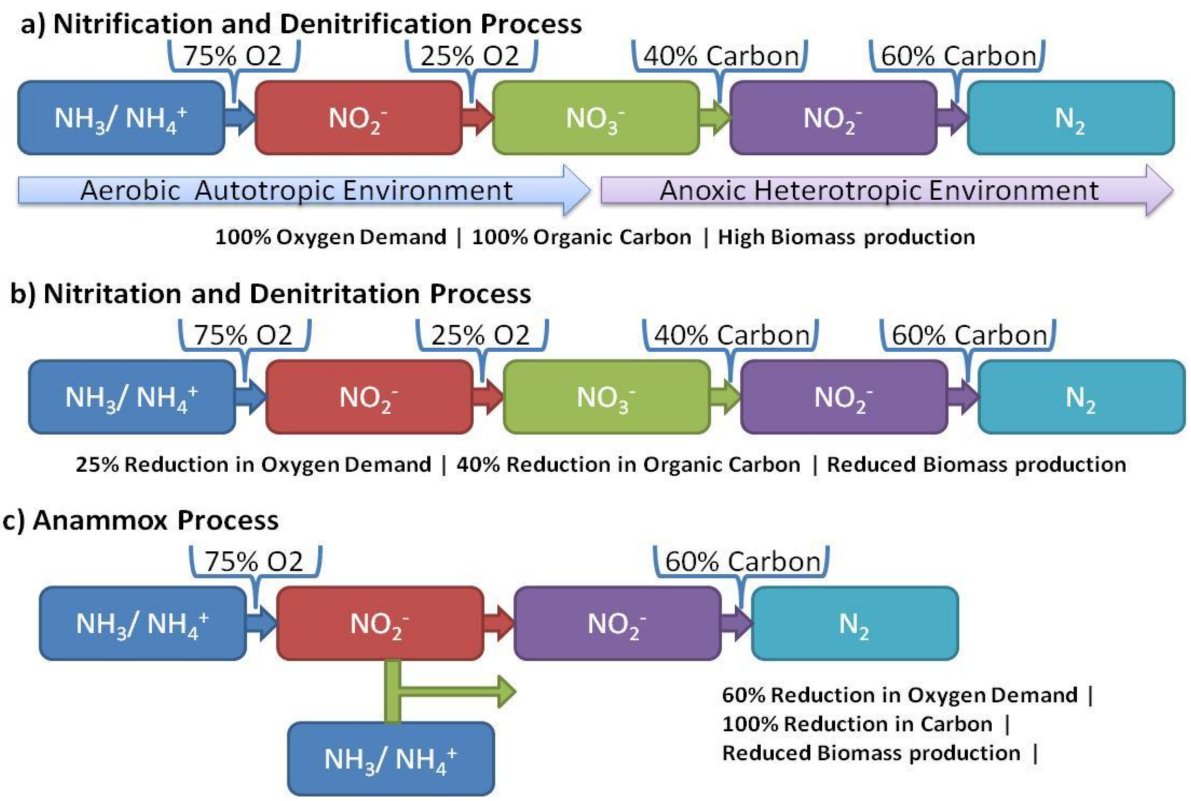

Figure 1: Biological nitrogen removal reactions: a) Conventional biological nitrification and denitrification reaction, b) Nitrogen removal reaction by nitritation- denitritation process and c) Nitrogen removal reaction by ANAMMOX process

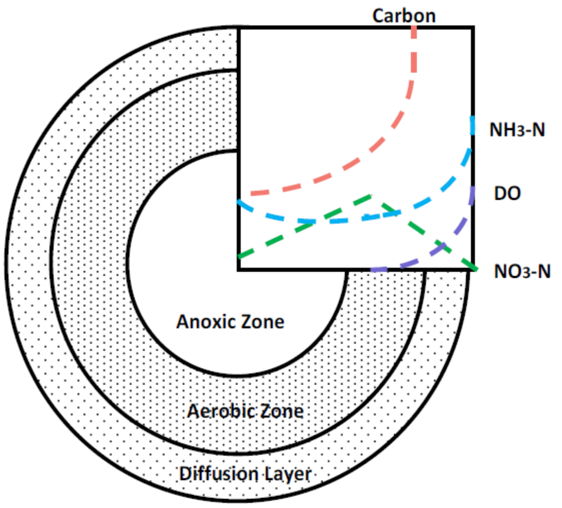

Figure 2: Diffusion of DO and substrate within the floc during SND process[24]

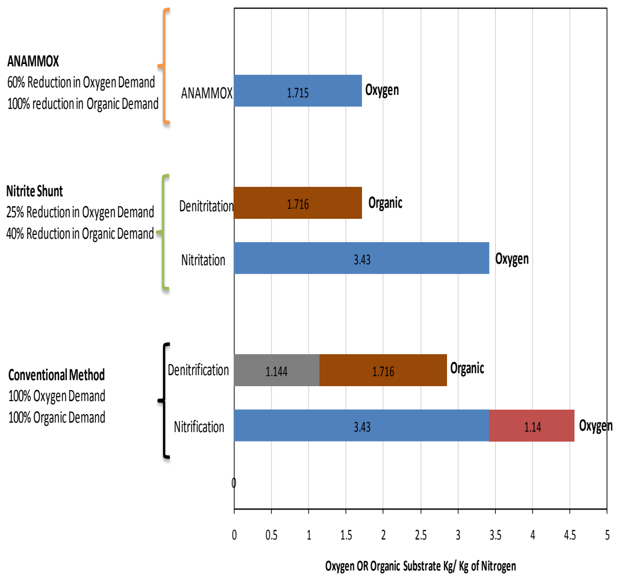

Figure 3: Comparison of oxygen and organic demands of different biological nitrogen removal processes

It is imperative to compare the significance of advance processes such as nitritation-denitritation and ANAMMOX with conventional processes. It is evident from figure 3 that the advance processes show considerable reduction in demand of oxygen and organic substrate. The oxygen requirement for nitrification of ammonia is 4.57 g O_2 /g N oxidized (3.43 g O_2/ gm of Ammonia oxidized to nitrite and 1.14 g O_2 / g NO_2 oxidized to nitrate).[63] Produced nitrate is further denitrified by heterotrophic microbes with the stoichiometric requirement of 2.86 g COD/g N[44] whereas the nitritation- denitritation process requires 1.761 g COD/g N of organic. The ANAMMOX process further reduces the oxygen requirement by 60% with no need of organic substrate.

The current approach should be towards use of low oxygen and carbon utilizing systems which are beneficiary for saving energy and avoiding environmental pollution.

Engineered biological nitrogen removal processes: Based on the known reactions many systems were engineered and applied for advance nitrogen removal in wastewater treatment. There are two major types of microbial growth process available in biological wastewater treatment which are a) biofilm based and b) granular sludge based, however, recent development also moved towards with c) a hybrid process which uses both biofilm and sludge based approach to treat the wastewater (Figure 4).

Biofilm based processes: Technologies such as Submerged Aerobic Fixed Film Reactor (SAFF), Trickling filters and biofilters are commonly known as fixed film processes in wastewater treatment. A nitrogen removal study from centrate type of wastewater with the use of a submerged attached growth bioreactor has shown 85% of average total nitrogen removal[45]. A recent study showed the nitrogen removal of 52–54% was reached in a partial nitritation in sponge-bed trickling filters (STF)[54]. Also, a report shows the enhanced nitrogen removal over 60% in conventionally designed trickling filter plants[9].

In the tertiary denitrifying biofilters, the studies show up to 82% of nitrate nitrogen (NO_3-N) removal[55]. A biofilter containing ammonia-oxidizing bacterial (AOB) and nitrite-oxidizing bacterial (NOB) communities was studied and resulted in 59.8–82.1% of total N removal efficiencies[67]. However, fixed film reactors are inadequately used for nitri-denitrification because of the difficulty in maintaining an anoxic environment.

The fluidized bed bioreactors or moving bed bioreactors (MBBR) and biofilm based air-lift reactor were reported in 1994 for nitrification[19]. In the evolution of MBBR process development, studies for nitri-denitrification, cold climate nitrogen removal, biofilm carriers and oxygen addition[4], sequencing batch MBBR, biological nitrogen removal[18] were established.

Further biofilm based study includes nitrification in biofilms[60], SRT and biofilm growth with suspended biomass in biofilm reactors[61], biofilm detachment study[14]. Effects of varying substrate loading rates, influence of dissolved oxygen concentration on nitrite accumulation[47], mathematical modeling of biofilm structures[41] have been accomplished for development of modern wastewater treatment methods.

The effect of ammonical nitrogen loading rate from 0.2 to 0.4 kg NH_4-N/(m^3.d) was studied on Kaldnes K1 and Mutag Biochip type of carrier media and resulted with total nitrogen removal amounting up to 86 and 73% respectively[6]. Cubic-shaped polyurethane sponges were also used as biofilm carriers in a MBBR and achieved total nitrogen removal up to 86.7% and correspondingly, SND was 93.3 %[71]. The application of conventional MBBR processes for nitrification and nutrient-removal is shown in figure 7.

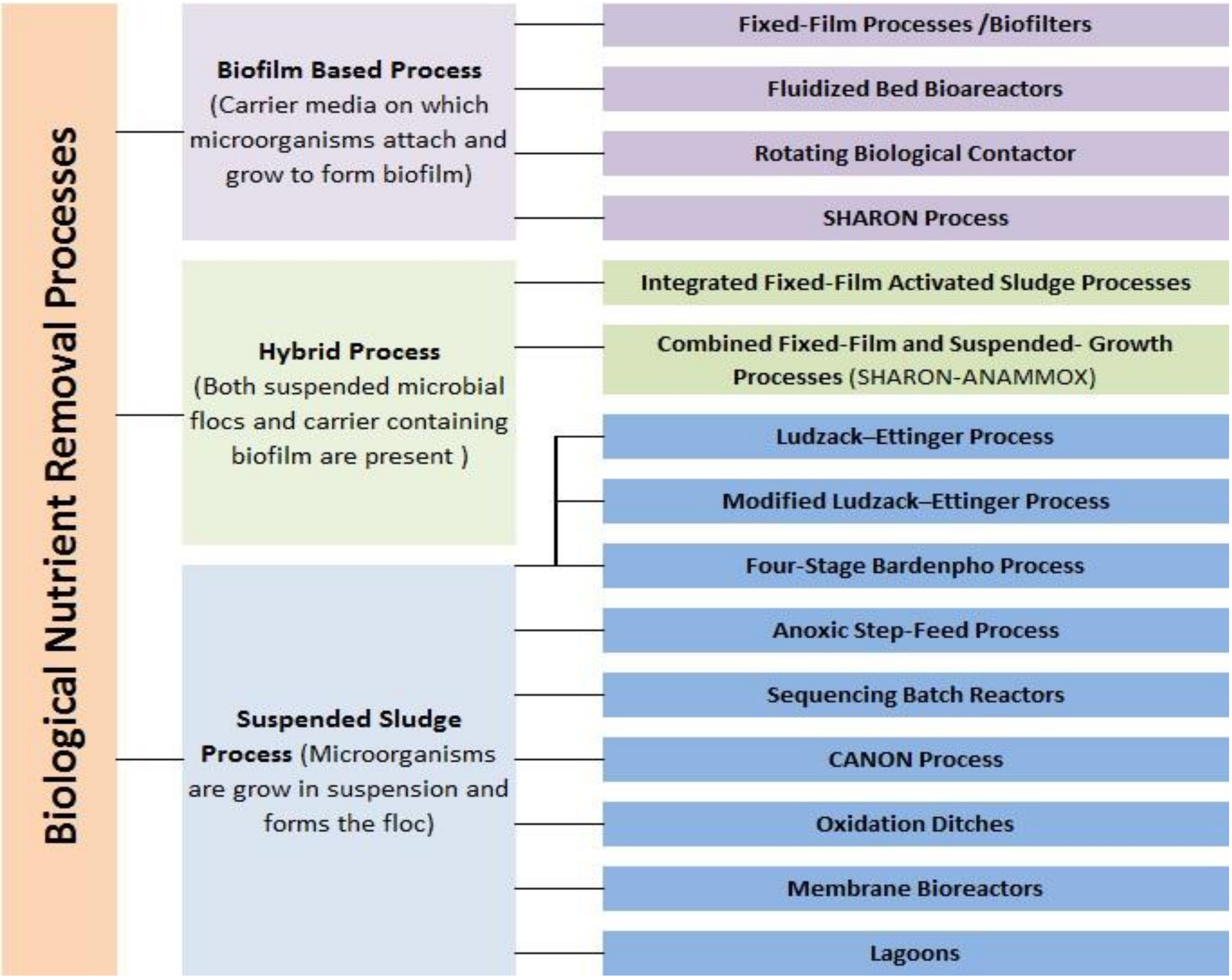

Figure 4: Current applied processes and technologies for biological nitrogen removal.

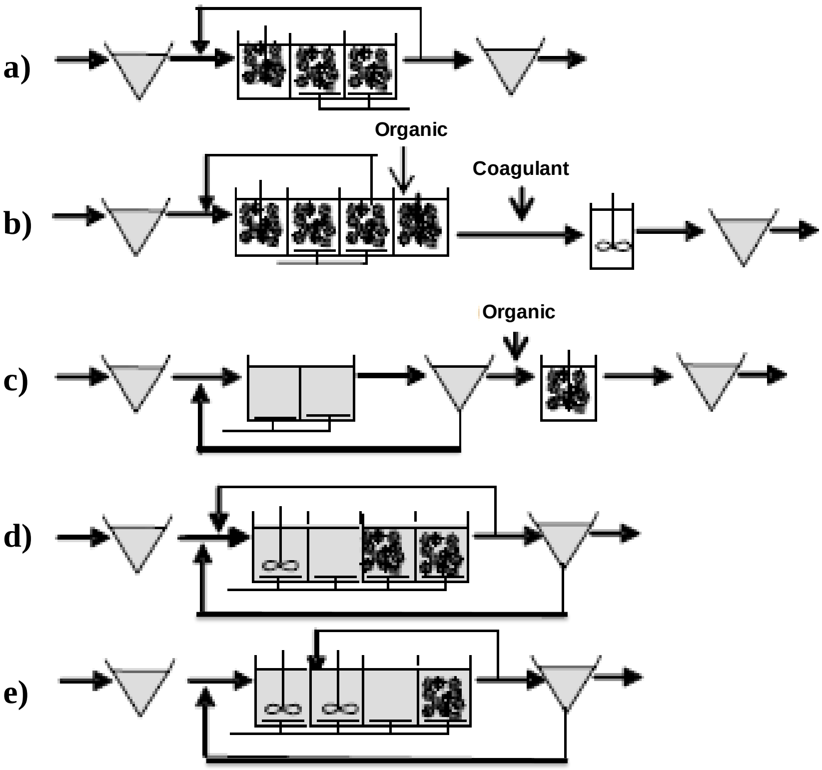

Figure 5: Flow diagrams for MBBR and IFAS processes for nitrogen removal applications[43]

MBBR processes are often preferred in green-field plants for nitrogen removal in A_2O (Anoxic- Oxic-Oxic) mode (Figure 5a). Sometimes to achieve more stringent nitrate removal pre-anoxic reactor with internal recycling (IR) followed by post-anoxic reactor with external carbon addition is often practiced (Figure 5b) in order to achieve effective denitrification. Anoxic MBBR can be used after nitrifying activated sludge plants as post-denitrification tool with external COD addition (Figure 5c) for denitrification. The SHARON process (single reactor system for high ammonia removal over nitrite process) is a biofilm based process of biological nitritation- denitritation (Figure 6a).

To obtain these stable reactions, the operating variables (temperature, pH, hydraulic retention time, substrate concentration, dissolved oxygen) are controlled in a chemostat operation with carrier media and stirrer[17]. But unfortunately, controlling of these process variables can be very difficult in large scale plant operations[57].

Hybrid processes: Recent study of Integrated Fixed-Film Activated Sludge (IFAS) MBBR technology has been explored to optimize various operating conditions by combining biofilm systems with activated sludge process and WAS recycling[12]. IFAS process enables activated sludge systems to achieve higher treatment efficiency without increasing MLSS concentration in the process. A performance evaluation study for biological nitrogen removal in an integrated fixed-film activated sludge and moving-bed sequencing batch biofilm reactor (IFAS-MBSBBR) obtained overall nitrogen removal efficiency up to 91.4%[48] to 91.7%[58].

Combined SHARON and ANAMMOX process (Figure 6b) also practiced where the effluent from SHARON reactor containing a mixture of ammonium and nitrite is ideally suited as the influent for the ANAMMOX process where ammonium and nitrite are anaerobically converted to dinitrogen gas by ANAMMOX bacteria[31]. Again, the requirements of higher nitrogen load and stringent process control are restrictions of this process. MBBR-based IFAS processes are mostly used for up-gradation of activated sludge plant in order to achieve total nitrogen removal (Figure 5d) or total nutrient (N and P) removal (Figure 5e). A sewage treatment study of ANAMMOX - MBBR and ANAMMOX – IFAS processes was attempted to compare the efficiency of biofilm based competitive reactions of nitritation/ANAMMOX biofilm in presence of granular sludge[30]. Figure 8 shows the ANAMMOX biofilm process with MBBR and IFAS based approach.

Lower C/N ratio is a pre-requisite of de-ammonification reactions. The de-ammonification process in MBBR (Figure 7a) uses the suspended carriers which reduce the foot print of the system. But, the diffusion limitation of NO_2-N in the biofilm may occur for ANAMMOX bacteria which will be possible rate limiting step in MBBR ANAMMOX process. The first full scale demonstration plant was built in Hattingen in Germany and was implemented as IFAS-based ANAMMOX process (Figure 7b) designed in such a way that the aerobic ammonium-oxidizing bacteria are enriched in the suspended activated sludge and the anaerobic ammonium-oxidizing (ANAMMOX) bacteria are enriched on a biofilm.

The side stream process at Sjölunda WWTP (Sweden) used IFAS – ANAMMOX process for treating reject water from sludge digestion with a removal capacity exceeding 2 kg $N/m^3.d$[8]. Till date 100 full-scale installations are in operation worldwide with partial nitritation/ANAMMOX (PN/A) process. All were implemented and optimized for high-strength ammonium wastewaters with low C/N ratios and elevated temperatures[32].

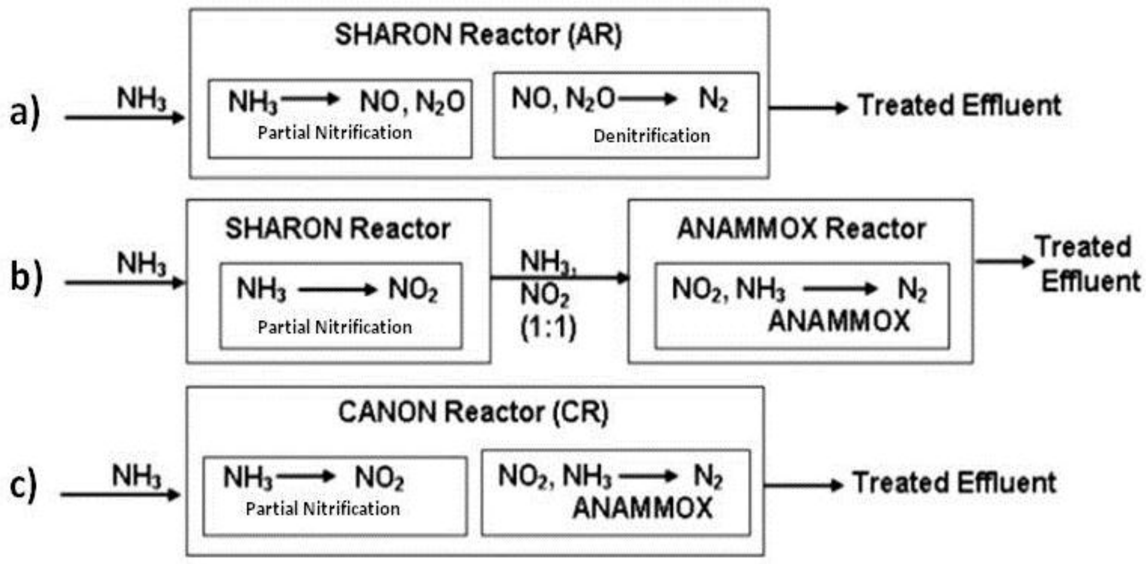

Figure 6: Process block diagrams of advanced biological nitrogen removal processes, namely the (a) SHARON process, (b) SHARON-ANAMMOX process, (c) CANON process[5]

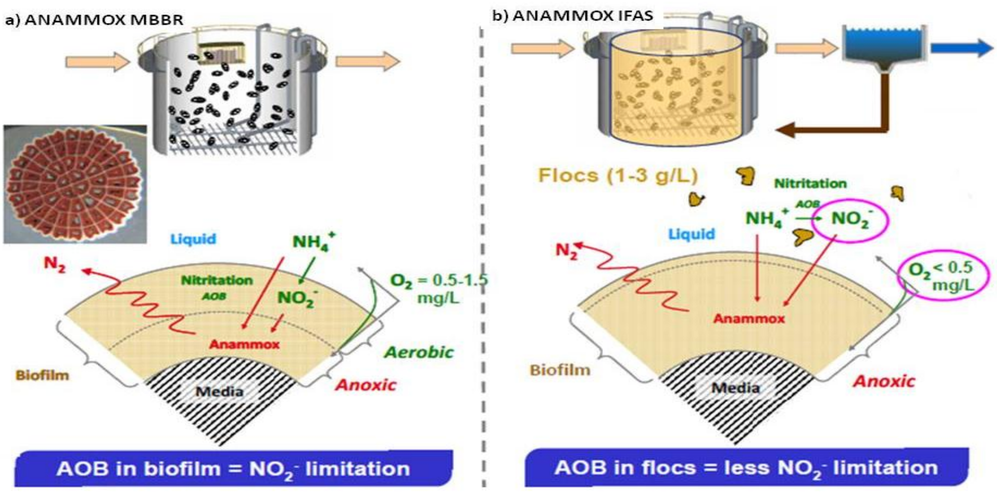

Figure 7: ANAMMOX reactions with MBBR and IFAS processes[8]

Although ANAMMOX process is showing great advantages in terms of energy saving in nitrogen removal, the process sensitivity and controlling parameters to effective reactions are current big challenges to encounter. Some impacting parameters such as pH shock, temperature variation, influent solids concentration and mixing problems are interrupting the plant performance. Some process controlling issues in actual ANAMMOX based nitrogen removal plants are: a) 30% plants have NH_4^+ build up issues, b) 50% plants have NO_3 and NO_2 build-up issues, c) 30 - 35% plants have scaling and foaming issues and d) 45% plants have sludge retention/settling/solids separation issues[32].

It has been reported that growth of ANAMMOX bacteria can be inhibited due to inhibition of the ammonium oxidizing bacteria and subsequent ammonia increase in reactor[27]. ANAMMOX process is effectively applied for nitrogen removal in various plants but further optimizing operational conditions need to be focused for increasing its practical sturdiness.

Suspended sludge processes: The conventional nitrogen removal processes such as Ludzack–Ettinger, Modified Ludzack–Ettinger (MLE), Four-Stage Bardenpho and Anoxic Step-Feed Processes are based on activated sludge philosophy with larger reactor volumes but they are very adaptable to retrofit in existing activated sludge process (ASP). In these systems, lack of fine control on internal recycle flow with anoxic DO control is the major limitation to achieve efficient denitrification. The pre- and post-denitrification plants (Figure 8) were studied by Rusten and Ødegaard stating that the parameters such as DO concentration, NO_x-N (NO_2-N + NO_3-N) concentration, concentration of organic matter and operating temperature influence the denitrification process[55]. Another study also concluded the dissolved oxygen of internal recirculation loop significantly reduces the anoxic denitrification rate[64].

Efficient total nitrogen removal in a pre-denitrification process is a function of internal recirculation ratios where it is typically limited to 100 to 200% of the influent flow depending on temperature and wastewater composition. The continuous operation of IR pump also incurs the additional cost to the overall treatment. In post-denitrification process, the addition of external carbon source such as methanol or ethanol is necessary.

Due to the significantly shorter start-up time and higher denitrification rates, ethanol will be the best external carbon source for post-denitrification in geographic markets where the ethanol is cost competitive; otherwise addition of external carbon source increases the operating cost[43]. The external carbon source addition needs to be controlled constantly to achieve the effective denitrification and to avoid surplus organic buildup. These pre- and post-denitrification processes also need higher footprint and trains of separate chambers to carryout reactions in separate zones.

Sequencing batch reactor (SBR) is a current alternate to activated sludge process which is gaining popularity due to its high-quality treatment performance and complete possible automation. SBR process consists of four steps namely feeding, aeration, settling and discharge of the treated effluent. It is an advanced cyclic activated sludge process which is working on typical batch process carrying SND phenomenon at different cycle phase[36].

In certain cases, multiple SBR reactors are used simultaneously for continuous treatment operation and at least one reactor is fed at each moment. SBR is a known tool for biological carbon and nutrient removal, capable of achieving effluents with very low nitrogen and phosphorus concentrations from highly concentrated wastewaters[42]. At feed phase, reactor meets high BOD and zero DO condition which is suitable for denitrification and become anaerobic for phosphate release. In the aeration phase, phosphate uptake reaction is followed by COD oxidation and nitrification (Figure 9). This SBR cycle design has the advantage of selector phase biology[36].

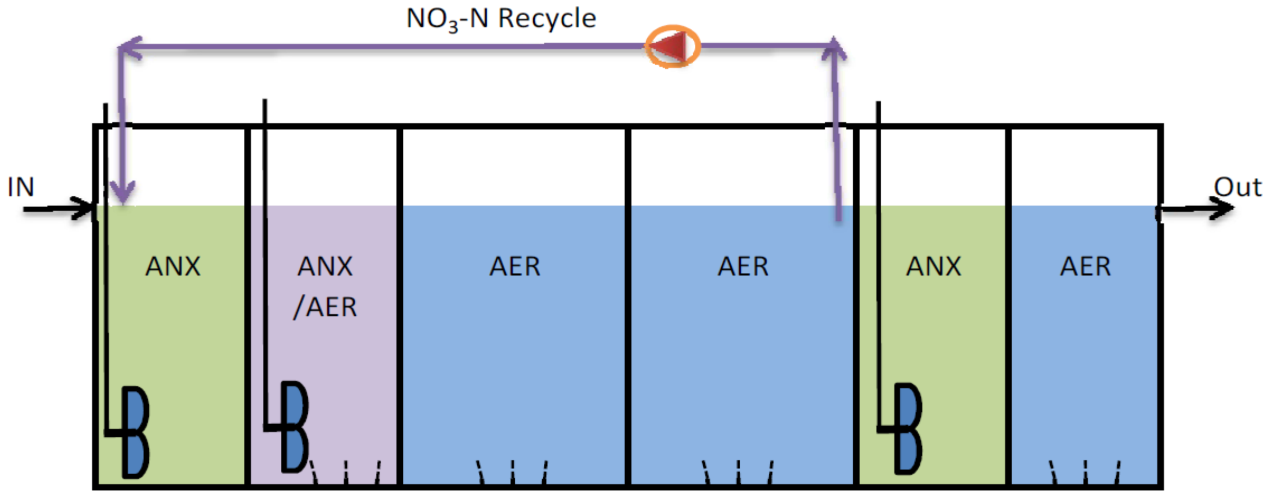

Figure 8: Nitrogen removal in combined pre- and post-denitrification MBBR process

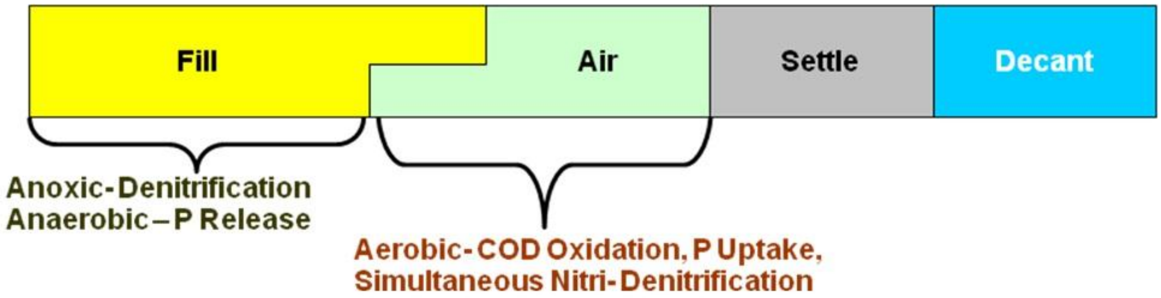

Figure 9: SBR cycle design for the advantage selector phase biology[36]

Use of ANAMMOX process with SBR operation philosophy was also attempted such as CANON SBR process. CANON (completely autotrophic nitrogen removal over nitrite) process (Figure 6c) relies on a stable interaction between the two groups of autotrophic microorganism populations where the nitritation and ANAMMOX reactions are carried out under oxygen limited conditions[59]. SBR was operated with an ammonium-rich wastewater with CANON process and achieved nitrogen removal rate of up to 0.3 kg $N/m^3.d$[56]. Slow reaction rate, controlling biomass population and strict DO control (< 0.5 mg/l) are the limiting factors of this process.

A recent study showed the simultaneous partial nitrification, ANAMMOX and denitrification processed effectively carried out in a SBR[69]. SBR is the easy batch process with need of longer reactor HRT and overall higher footprint where it might require effluent equalization before filtration. Also, SBR needs higher sophisticated instrumentation to handle complex process design with reliable decanting system.

Although the present Nitritation/ ANAMMOX process is known for most efficient way of nitrogen removal, it demands and is restricted to its reaction requirements of higher HRT, lower C/N ratio, higher influent ammonium concentration[26,32]. ANAMMOX process for nitrogen removal from sewage at higher C/N ratio is not yet well established. In the case of nitritation/denitritation study, the usefulness of an intermittent aeration strategy for achieving nitrite accumulation remains unknown[46]. The one-step partial nitritation/ANAMMOX or de-ammonification process is challenging to control because of the competing reactions of ANAMMOX, ammonia-oxidizing (AOB), nitrite-oxidizing (NOB) and denitrifying bacteria that reside in a bioreactor[39].

Further study should focus on determining process control parameters for the optimal operation of nitritation-ANAMMOX bioreactors under variable wastewater conditions such as lower temperature and high C/N ratio[21]. The process parameters required to control the energy efficient nitrogen removal are still not achieved to its possible optimum. Higher HRT, lower C/N ratio, controlling internal recycle and stabilizing different bacterial population on actual plant conditions are some of parameters needed to consider for further research.

Modeling of biological nitrogen removal processes: Biological nitrogen removal process modeling is a current requisite for better process selection, treatment predictions and gives a platform for better management of treatment responses in a short time. A series of activated sludge models (i.e. ASM1, ASM2, ASM2d, ASM3) developed by International Water Association (IWA), have been considered as good tools for correlating the complexity of the activated sludge processes and the prediction of biological treatment efficiency under dynamic conditions. A more complex ASM2d and ASM3 has occurred to describe the biological nitrogen removal processes[68]. Many factors such as HRT, % removal, air flow rate, MLSS in the reactor, SRT and organic or nitrogen loading rates are to be analyzed in these models to achieve optimum process parameters.

The mathematical and program based modeling tools are also available to understand the complex interdependent biological processes. Results of a single air lift bioreactor carrying SND process were completely analyzed using analysis of variance (ANOVA) by Design Expert software. From this analysis, three-dimensional plots were obtained based on the effect of the levels of the two factors and the simultaneous interaction of the two factors on the treatment responses[3]. The comparative studies with activated sludge model (ASM)[10] and one-dimensional multispecies biofilm reactor model[23] have given better understanding of simultaneous nitrogen removal process reactions. In a model based evaluation of shortcut nitrogen removal processes, the two control schemes were evaluated; one was based on online measured ammonia and the other was based on a target ratio of ammonia vs. NO_x (AVN). The results promote better management of incoming organics and bicarbonate for a more efficient NOB out-selection[1].

Kagawa et al[28] coupled two computational models for biological nitrogen removal study in the SBR with anaerobic– aerobic–anoxic cycles with different spatial and temporal scales. The results of this study indicated the stability of nutrient removal process using microbial granules depending largely on the DO concentration during the aerobic period. Regmi et al[50] evaluated the aeration control strategies: 1) DO setpoint control or conventional aeration control (CAC); 2) Ammonia-based aeration control (ABAC); and 3) AVN control. After experimentation, the comparison of these strategies in terms of effluent total nitrogen concentrations, aeration demand, alkalinity demand nitrite-shunt was done and the results showed the insights on the advantages and limitations of each aeration control strategy. This type of modeling analysis completely avoids the time consuming wet or pilot experimentations.

Huang et al[22] compared the ANAMMOX based nitrogen removal experimental performance, with Monod model modified Stover–Kincannon model, first-order kinetics and the Grau second-order substrate removal model. Based on analysis, Monod model, modified Stover–Kincannon model and the Grau second-order model proved to be more appropriate to describe the nitrogen removal kinetics of the reactor than first-order model with high determination coefficients of 0.993, 0.993 and 0.991 respectively. Applications of these models will give rapid understanding with thorough comparative analysis of the different biological nitrogen removal processes. So, there is a need for further advancement of bioprocess based nitrogen removal models which can be easily applied towards precise selection of process parameters and modification with more feasible and cost-effective way.

Conclusion

To evade eutrophication for river protection and other water bodies, nitrogen removal need should be mandatory for every wastewater treatment facility. The review highlights the conventional methods and processes that are practiced for the purpose of BOD and nitrogen removal, pay higher energy cost and also capital intensive. The most popular biological process technologies such as high rate biofilm based MBBR, hybrid suspended growth - biofilm based IFAS and suspended growth based MLE and SBR are the current choices to aim the nitrogen removal. But these processes follow the conventional biology of nitrification and denitrification where each reaction incurs the cost to create separate environment.

A need exists to evolve new process for nitrogen removal which should be more energy neutral and cost effective through exploiting current knowledge and smart engineering.

In this sphere, the granular sludge process technology opens the promising space with simultaneous nitri-denitrification (SND) mechanism and creates scope for further research in varied areas such as achieving sludge granulation in actual plant conditions, controlling granule size, achieving CSTR conditions, maintaining low DO, sludge recycling and lowering the overall footprint.

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