ISBN: 978-628-96613-3-0. ISSN: 2414-6390. Digital Object Identifier: https://dx.doi.org/10.18687/LACCEI2026.1.1.1781

Polyvinyl Alcohol-Sodium Alginate-Zeolite Biofilm Carriers to Enhance Wastewater Deammonification

Sheyla Chero-Osorio[1,3]; Cooper James[2]; Susieanna N. Persaud[2]; Ananda S. Bhattacharjee[1]; John N. Kuhn[2]; Sarina J. Ergas[1]

[1]Department of Civil and Environmental Engineering, University of South Florida, United States of America, *sheylac@usf.edu, asbhattacharjee@usf.edu, sergas@usf.edu*

[2]Department of Chemical, Biological and Materials Engineering, University of South Florida, United States of America, *cooperjames@usf.edu, persaud193@usf.edu, jnkuhn@usf.edu*

[3]Centro de Investigación y Tecnología del Agua – CITA, Universidad de Ingenieria y Tecnologia – UTEC, Peru, *schero@utec.edu.pe*

Abstract– ***Polyvinyl alcohol-sodium alginate (PVA-SA) hydrogel biofilm carriers exhibit high biomass retention and porosity. In this study, novel composite PVA-SA carriers were synthesized for partial nitritation/anammox (PN/A) by encapsulating a zeolite mineral (chabazite) and anammox inside the carrier and growing ammonia oxidizing microorganisms (AOMs) on the carrier surface. Initial experiments were carried out in anaerobic sequencing batch reactors (SBRs) with an anammox-based feed. Limited ammonium removal was observed in abiotic controls, while SBRs containing carriers or suspended cultures simultaneously removed ammonium and nitrite, while generating nitrate. AOMs were subsequently inoculated into the SBRs and feed and aeration conditions were adjusted to promote PN/A. Nitrate generation was lower in the carrier SBR compared with suspended growth control, resulting in total inorganic nitrogen removal rates of 11.3 mg N/L/d and 8.9 mg N/L/d for carrier and suspended SBRs, respectively. Overall, the PVA-SA-chabazite carrier SBR exhibited enhanced PN/A performance, most likely because chabazite concentrated ammonium inside the carriers to promote mass transfer and enhance anammox activity. Chabazite was bioregenerated by AOMs and anammox, allowing the carriers to be reused for fifteen cycles over 185 days. The results underscore the potential application of novel PVA-SA-chabazite carriers to promote PN/A for wastewater treatment.***

Keywords-- ***Ammonium Adsorption, Bioregeneration, Chabazite, Partial Nitritation/Anaerobic Ammonium Oxidation, Zeolite-Embedded Hydrogels.***

I. Introduction

Conventional biological nitrogen removal (BNR) processes have high energy and chemical requirements for nitrification/denitrification[1]. Partial nitritation/anaerobic ammonium oxidation (anammox), also known as PN/A or deammonification, has the potential to eliminate organic carbon addition, reduce oxygen demand by 60% and reduce excess sludge production by 80%[2]. However, application of PN/A for domestic wastewater treatment faces challenges due to slow growth rates of anammox and ammonia oxidizing microorganisms (AOMs) and competition for substrates by nitrite-oxidizing bacteria (NOB) and heterotrophic denitrifiers [2]. Integration of PN/A in biofilm reactors allows for retention of slow-growing microorganisms. Biofilm reactors can also be operated to promote low dissolved oxygen (DO) outer layer favoring AOMs and an anoxic inner layer favoring anammox [3].

Natural zeolite minerals, such as chabazite, are commonly used as ion exchange (IX) resins because of their capacity and selectivity for ammonium [4]. Several prior studies have shown that zeolite-based biofilm carriers can enhance PN/A activity by separating and concentrating ammonium from wastewater [5]. Zeolite has previously been incorporated into organic polymeric biofilm carriers. For example, polyethylene carriers were coated with faujasite, resulting in a 75% ammonium removal efficiency from synthetic wastewater with 45 mg/L NH_4^+-N [6]. Composite carriers for a moving bed biofilm reactor (MBBR) were developed by combining zeolites and floating foam materials; nitrogen conversion rates for zeolite-based carriers were 33% higher than for unmodified carriers [7].

Hydrogels are hydrophilic polymer-based structures that have been used as biofilm carriers [8]. The elastic and flexible nature of hydrogels enables swelling, while the crosslinked polymer chains provide structural integrity [9]. Different types of gel carriers have been used for anammox, including polyvinyl alcohol (PVA) only, sodium alginate (SA) only, PVA-SA and sodium carboxymethylcellulose [10]. However, PVA-SA is considered the best material for biological nitrogen removal because of its spatial structure and cost [10]. Integration of PVA with SA promotes mass transfer, enhances mechanical strength, and allows the material to be shaped into different forms [11].

PVA-SA has been used to produce semi-permeable hydrogel biofilm carriers that provided the anaerobic environment needed by anammox [12], leading to improved nitrogen removal [13]. A recent study integrated zeolites and denitrifying bacteria into PVA-SA carriers for river water treatment [14]. However, no prior studies have integrated natural zeolites and anammox into PVA-SA biofilm carriers for PN/A.

In this proof-of-concept study, chabazite was integrated into PVA-SA hydrogels (PVA-SA-chabazite) to create novel biofilm carriers for PN/A. Anammox bacteria were encapsulated inside the PVA-SA-chabazite carriers while AOMs formed biofilms on the carrier surface (Fig. 1). The guiding hypothesis was that PVA-SA-chabazite carriers create regions with high ammonium concentrations to promote mass transfer and enhance substrate availability for PN/A activity.

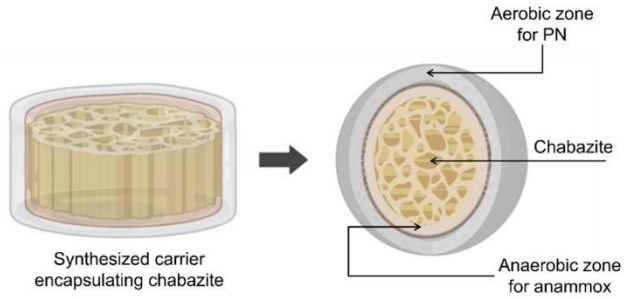


Fig. 1 Synthesized PVA-SA-chabazite carrier for PN/A, promoting an aerobic outer layer for PN and an anaerobic inner layer for anammox. Addition of chabazite creates high ammonium concentration regions.

The objectives of this study were to: (1) develop an effective protocol for fabricating PVA-SA-chabazite carriers, (2) evaluate the effect of chabazite addition on abiotic ammonium removal by PVA-SA-chabazite carriers, (3) compare nitrogen transformations in sequencing batch reactors (SBRs) containing PVA-SA-chabazite carriers with suspended growth controls, and (4) evaluate bioregeneration and reuse of the PVA-SA-chabazite carriers by PN/A biofilms using synthetic wastewater and pretreated municipal wastewater.

II. Materials and Methods

Experiments were conducted in SBRs over four phases (Table I) to evaluate the performance of fabricated carriers and controls under varying conditions. Additional details can be found in [15].

TABLE I
Experimental conditions for the SBRs over four phases

Phase	Purpose	Feed N Concentrations	Aeration conditions	Days of operation
1	Anammox comparison with abiotic controls	NH_4^+-N: 120 mg /L NO_2^--N: 144 mg /L	Anaerobic	0-49
2	Long-term anammox activity			49-119
3	PN/A synthetic feed	NH_4^+-N:120 mg/L	Aerobic	119-159
4	PN/A municipal wastewater	CEPT wastewater (NH_4^+-N: 40 mg/L)		159-185

CEPT = chemically enhanced primary treatment

A. Feed Composition

Synthetic wastewater for Phases 1 through 3 was prepared using local groundwater (Tampa, FL, US), with added buffer and trace element solutions [15], [16]. Ammonium carbonate and sodium nitrite were purchased from Fisher Scientific (Waltham, MA, US) and added to achieve target N species concentrations shown in Table I. The feed for Phase 4 was prepared to resemble municipal wastewater after chemically enhanced primary treatment (CEPT) [5]. Briefly, influent was collected from a local wastewater treatment facility (Tampa, FL, US) and mixed with groundwater (60% v/v) to reduce solids and chemical oxygen demand (COD) concentrations. Ammonium bicarbonate was added to achieve a NH_4^+-N concentration of 40 mg/L.

B. Materials for Carriers

AZLB-Na Bowie Chabazite was obtained from the St. Cloud Mining company (Winston, NM, US). Chabazite pretreatment was done to enhance ammonium exchange by soaking in local groundwater for 3 h and rinsing with DI [17]. Pretreated chabazite was crushed and sieved to a particle size between 0.1-0.4 mm. PVA (Mw 89,000-98,000, 99+% hydrolyzed) and SA were purchased from Sigma-Aldrich (St. Louis, MO, US). Barium chloride ($BaCl_2$) solutions (4% w/w) were prepared from anhydrous barium chloride (≥97%) (Fisher Scientific, Waltham, MA, US). Disk silicone molds (4 cm diameter x 2 cm depth each hole) were purchased from Baker Depot (Zhejiang, China).

Enrichment reactors for PN/A were set up separately in a 22 °C constant temperature room. Enrichments for AOM and anammox were maintained for three and four years and monitored based on nitrogen species performance. Details on the enrichments can be found in [18].

C. Carrier Fabrication

Fabrication of PVA-SA-chabazite carriers is shown schematically in Figure 2. SA (0.5 g) was added to a 50 mL beaker, followed by addition of PVA (0.5 g) and DI water (7 g). The mixture was placed on a heating mantle at 120°C, stirred for approximately 1 h and then transferred to a second beaker. Chabazite and enriched anammox biomass were added to the PVA-SA mixture and then transferred to the silicon molds. Three different types of PVA-SA-chabazite carriers were fabricated (Fig. 2): (a) no chabazite, (b) with chabazite (1 g) and (c) with both chabazite (1 g) and enriched anammox biomass (2 mL). A $BaCl_2$ crosslinking solution (4% w/w) was added to the molds. Molds were covered with parafilm for 24 h to complete crosslinking. The remaining $BaCl_2$ solution and DI were decanted out.

D. SBR Setup and Operation

Four SBRs were set up in 500-mL Erlenmeyer flasks: (a) abiotic control containing a PVA-SA carrier without chabazite or biofilm (PS), (b) abiotic control containing a PVA-SA carrier with chabazite (PSZ), (c) suspended growth control with 2 mL of enriched anammox biomass (suspended), and (d) experimental SBR containing a PVA-SA carrier with encapsulated chabazite and anammox (carrier). SBRs were incubated in a constant temperature room at 22 °C.

During Phases 1 and 2, anaerobic conditions were provided by sealing the flasks with rubber stoppers and connecting the reactor headspace to 1-L SKC FlexFoil gas bags (Eighty Four, PA, US). During Phase 2, only the suspended and carrier SBRs were operated for four fill and draw cycles to compare long term anammox performance with

and without the PVA-SA-chabazite carriers under anaerobic conditions.

At the beginning of Phase 3, enriched AOM biomass (1 mL) was added to the suspended and carrier SBRs to investigate PN/A performance. During Phases 3 and 4, SBRs were unsealed and placed on a VWR Advanced Digital Shaker (Frederick, MD) at 60-70 RPM to promote microaerophilic conditions. SBRs were replenished with fresh feed when 90% ammonium removal efficiency was observed.

E. Analytical Methods

All analytical methods followed *Standard Method* protocols [19]. Cation and anion (NH_4^+, NO_2^- and NO_3^-) concentrations were measured using a 881 Compact Pro Ion Chromatography system (Metrohm AG, Herisau, Switzerland). Method-detection limits for NH_4^+, NO_2^-, and NO_3^- were 0.30, 0.01, and 0.01 mg/L, respectively. pH and DO were measured at the beginning and end of selected cycles using Orion 5 Star (Thermo Scientific, Beverly, MA, US) and HQ440d (Hach, Loveland, CO, US) multifunction meters. COD was measured using Lovibond (Sarasota, FL, US) reagent vials and a Lovibond TR 500 spectrophotometer. Values in figures are presented as means of duplicates. Statistical significance was determined using *t*-tests in Microsoft Excel, with *p*-values <0.05 considered statistically significant.

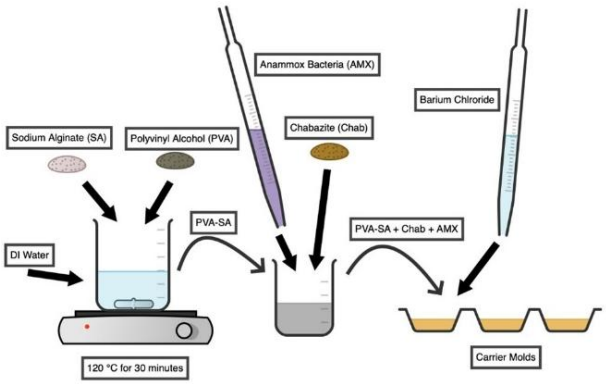


Fig. 2 Fabrication of carriers used for batch reactor experiments

III. Results and Discussion

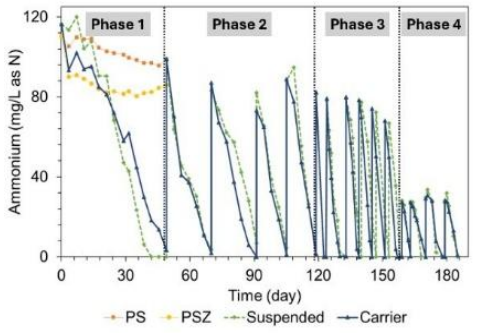
Concentration profiles for ammonium, nitrite, and nitrate for all experimental phases are shown in Figure 3.

A. Phase 1: Abiotic Controls and Anammox Acclimation

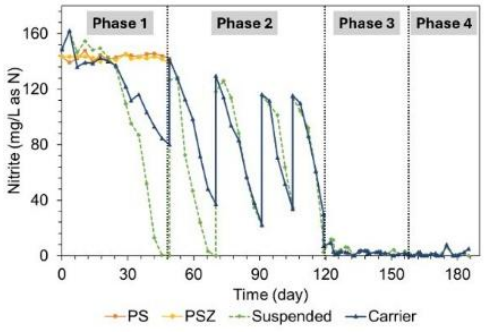
A small amount of ammonium was removed in the abiotic controls due to adsorption to the PS and PSZ surfaces (Fig. 3A). Ammonium adsorption capacities in the abiotic PS and PSZ reactors averaged 4.2 and 12.9 mg N/g, respectively. Negligible nitrite was removed (Fig. 3B) and nitrate was not produced (Fig. 3C) in abiotic controls.

For PS, ammonium removal was likely due to adsorption by the PVA-SA hydrogel. The adsorption of ammonium by hydrogels was previously reported to be mainly controlled by electrostatic attraction between ammonium and negatively charged hydrogels [20], [21]. Zheng et al. achieved ammonium removal in PVA-based optimized hydrogels, with the highest adsorption capacity of 42.7 mg N/g at 30 °C [20]. For PSZ, ammonium removal was due to adsorption onto the encapsulated chabazite until a constant concentration was reached due to chabazite saturation. The chabazite used in this study was previously evaluated using isotherm experiments. The maximum adsorption capacity of chabazite using low-strength wastewater (40 mg NH4+-N/L) was 22.5 mg N/g [18]. A possible explanation for the low adsorption capacity (12.9 mg N/g) of the abiotic PSZ carrier compared to that of natural chabazite is the limited ammonium mass transfer when chabazite was embedded in the PVA-SA hydrogel mixture.

In both the suspended and carrier SBRs, a lag period of 21±3.5 days was observed before ammonium and nitrite were removed simultaneously. The lag period was within the range previously reported for polymeric carriers with embedded anammox [22]. The suspended growth SBR exhibited greater nitrite removal than the carrier in the first SBR cycle (Fig. 3B), possibly because anammox were exposed to elevated temperatures during carrier fabrication. Decreases in ammonium and nitrite concentrations were accompanied by increases in nitrate concentrations, with greater nitrate accumulation in the suspended growth SBR than the carrier SBR (Fig. 3C). At the end of Phase 1, the NO_3^--N/NH_4^+-N ratio for the suspended growth SBR exceeded the theoretical stoichiometric ratio of 0.26 [23]. For the carrier SBR, the low nitrate concentration at the end of the first cycle indicated that the high ammonium concentration region in the chabazite, high nitrite concentration in the liquid phase, and anaerobic conditions within the carrier favored anammox outcompetition over NOB [24], [25], [26].



A)



B)

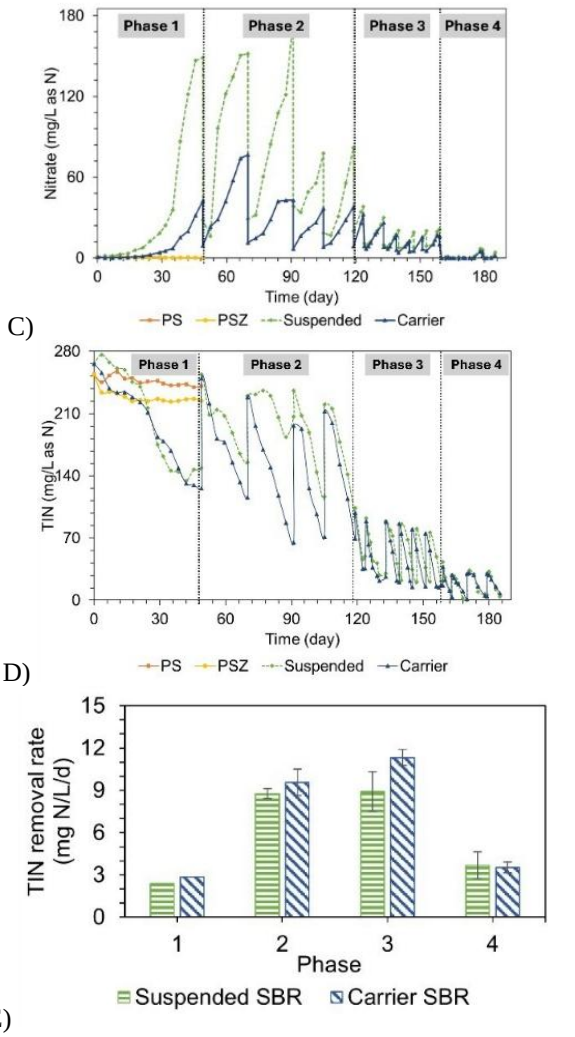


Fig. 3 Concentration profiles during Phases 1, 2, 3, and 4 for: (a) ammonium, (b) nitrite and (c) nitrate, (d) total inorganic nitrogen (TIN). (e) TIN removal rates for each phase (error bars show standard deviations).

TABLE II
TIN REMOVAL RATES FOR EACH PHASE

Phase	Purpose	TIN removal rate (mg N/L/d)	
		Suspended SBR	Carrier SBR
1	Anammox comparison with abiotic controls	2.4	2.9
2	Long-term anammox activity	8.8 ± 0.35	9.6 ± 0.93
3	PN/A synthetic feed	8.9 ± 1.40	11.3 ± 0.56
4	PN/A municipal wastewater	3.7 ± 0.95	3.5 ± 0.38

B. Phase 2: Long-term Anammox Performance

During Phase 2 (cycles 2-5), ammonium removal in the carrier SBR was slightly faster than for the suspended control (Fig. 3A), while nitrite removal in the suspended growth control surpassed the carrier SBR. Starting in Cycle 3, nitrite removal by the carrier was slightly faster than that by suspended growth (Fig. 3B). Similar to Phase 1, the decrease in ammonium and nitrite was accompanied by an increase in nitrate, with significantly higher nitrate accumulation in the suspended growth SBR. At the end of Cycle 3, SBRs and feed solutions were purged with N_2 gas prior to feed replenishment to reduce the potential for nitrate production by NOB activity. Although this reduced nitrate generation in both SBRs (Fig. 3C), the rate of nitrate accumulation in the suspended growth SBR continued to exceed the carrier SBR. Due to nitrate accumulation, average total inorganic nitrogen (TIN) removal rates at the end of Phase 2 were 8.8±0.35 and 9.6±0.93 mg N/L/d for the carrier and suspended growth SBRs, respectively (Fig. 3D, Fig. 3E, Table 2). Both the suspended and carrier SBR systems demonstrated stable long-term anammox activity, and no statistically significant difference was observed between the two systems ($p = 0.37$), indicating that the presence of the biofilm carrier did not negatively impact anammox performance under controlled conditions.

A concentration of 8.68 mg O_2/L in the feed before purging may have led to an increase in nitrate due to nitrification; however, the maximum amount of nitrate that could be produced was calculated as only 2.7 mg N/L. A possible reason for lower nitrate generation in the carrier SBR is that the carriers promoted anammox biofilm growth with an enhanced EPS matrix. It was found that hydrolysis of EPS into soluble compounds by anammox biofilms supported heterotrophic denitrification, even without external organic carbon addition [27]. In addition, nitrate reductase genes (*nar*) for nitrate reduction to nitrite have been found not only in heterotrophs but also in the anammox biofilm genome sequence [28], [29].

C. Phase 3: PN/A Performance with synthetic wastewater

During Phase 3 (cycles 6-11), SBRs were inoculated with AOMs, nitrite was removed from the feed and the SBRs were incubated under low DO conditions. The Phase 3 feed (pH = 8.20) had a free ammonia (FA) concentration of 8.2 mg/L, which was higher than the inhibitory threshold for NOB [24]. PN/A was successfully implemented in both bioreactors (Fig. 3), with no significant nitrite accumulation in either system. Under PN/A conditions with synthetic feed, the carrier SBR achieved a higher TIN removal rate (11.3±0.56 mg N/L/d) compared to the suspended SBR (8.9±1.40 mg N/L/d). Although this difference did not reach statistical significance ($p = 0.15$), a trend towards enhanced performance in the carrier SBR was observed, which may be attributed to the increased biofilm surface area provided by the PVA-SA-chabazite carrier.

During Cycle 7, DO concentrations ranged from 1.79 to 2.19 mg/L for the carrier SBR and 0.90 to 0.56 mg/L for the suspended growth SBR. The strategy of creating high ammonium regions in the chabazite-based carrier may have favored PN/A outcompetition over NOB, despite higher DO levels than those reported for stable anammox activity [30]. This finding emphasizes the suitability of a composite carrier that relies on distribution of AOMs and anammox on the outer

and inner surfaces [7]. For PN/A carriers encapsulating zeolite, Miladinovic and Weatherley [31] found that inoculated AOM utilized the ammonium adsorbed to the zeolite as substrate. High ammonium concentration regions and low oxygen conditions around the zeolites inhibited NOB proliferation and nitrate production and promoted anammox activity. Similar results were observed by Song et al. for simultaneous nitrification-denitrification using zeolite-based carriers [32]. Due to AOM biofilm growth on the carrier, DO was consumed, promoting the growth of anoxic microbial communities within the carrier layers.

D. Phase 4: PN/A Performance with semi-synthetic municipal wastewater

During Phase 4 (cycles 12-15), the feed was switched to semi-synthetic CEPT treated municipal wastewater. All other conditions were the same as in Phase 3. Similar ammonium and TIN removal rates were observed for both carrier (3.5 mg N/L/d) and suspended SBRs (3.7 mg N/L/d) (Fig. 3E, Table 2). No significant nitrite or nitrate accumulation were observed in either SBR. The Phase 4 feed had a low FA concentration of 0.9 mg/L, which was below the inhibition threshold for NOB [24]. An initial COD content of 150 mg/L was reduced to 40 and 30 mg/L in carrier and suspended SBRs, respectively. It was likely that organic matter in the semi-synthetic CEPT treated municipal wastewater contributed to heterotrophic denitrification [1].

When both suspended and carrier SBRs were fed with municipal wastewater, TIN removal rates dropped considerably and were virtually identical between the SBRs (3.7±0.95 vs. 3.5±0.38 mg N/L/d, $p = 0.81$). This suggests that the complexity of municipal wastewater composition may have limited the performance of both systems equally, and that further optimization of the carrier material and process conditions will be necessary to maintain removal efficiency under real-world conditions.

At the end of Phase 4 the carrier partially disintegrated (Fig. 4). PVA-based hydrogel carriers have been shown to be biodegradable, susceptible to carrier softening and partial microbial washout during long-term use in wastewater applications [33]. Future research should focus on mitigating carrier disassembly by adding high amounts of SA and sodium sulfate [34].

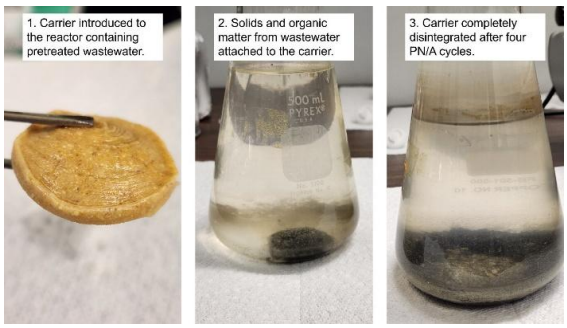


Fig. 4 Evolution of carrier disintegration after PN/A treatment of pretreated municipal wastewater

IV. Conclusions

This was the first study to encapsulate zeolite and anammox into PVA-SA hydrogel biofilm carriers for wastewater deammonification. Under synthetic feed conditions, lower NO_3^- generation and a trend towards higher TIN removal rates were observed in the Carrier SBR compared to suspended growth controls, although these differences did not reach statistical significance. When exposed to municipal wastewater, both systems exhibited comparable and reduced removal performance, highlighting the need for further optimization of carrier materials to resist biodegradation and maintain efficacy over long-term, real-world applications. These findings demonstrate the feasibility of this encapsulation approach and suggest potential for reducing chemical and energy costs of biological nitrogen removal upon scale-up.

Acknowledgment

The authors wish to thank Meng Wang, Sheldon Tarre, Michal Green, Huy Nguyen, Bhagyashree Kulkarni, and Tione Grant for their contributions to this project. This material is based upon work supported by the National Science Foundation under Grant Number 2000980, as well as the American Association of University Women (AAUW) International Fellowship 2022-2023.

References

[1] S. J. Ergas and V. Aponte-Morales, “Biological Nitrogen Removal,” *Comprehensive Water Quality and Purification*, vol. 3, pp. 123–149, Jan. 2014, doi: 10.1016/B978-0-12-382182-9.00047-5.

[2] Y. Cao, M. C. M. van Loosdrecht, and G. T. Daigger, “Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies,” Feb. 01, 2017, *Springer Verlag*. doi: 10.1007/s00253-016-8058-7.

[3] M.-K. H. Winkler, R. Kleerebezem, and M. C. M. van Loosdrecht, “Integration of anammox into the aerobic granular sludge process for main stream wastewater treatment at ambient temperatures,” *Water Res.*, vol. 46, no. 1, pp. 136–144, Jan. 2012, doi: 10.1016/j.watres.2011.10.034.

[4] V. J. Inglezakis, “The concept of ‘capacity’ in zeolite ion-exchange systems,” *J. Colloid Interface Sci.*, vol. 281, no. 1, pp. 68–79, Jan. 2005, doi: 10.1016/j.jcis.2004.08.082.

[5] S. Chero-Osorio *et al.*, “Ion exchange and bioregeneration by partial nitritation/anammox for mainstream municipal wastewater treatment,” *Bioresour. Technol.*, vol. 436, p. 132990, Nov. 2025, doi: 10.1016/j.biortech.2025.132990.

[6] E. C. Feinberg, A. L. Huff Chester, P. J. Novak, and M. A. Hillmyer, “Porous Polyethylene-Supported Zeolite Carriers for Improved Wastewater Deammonification,” *ACS ES&T Engineering*, vol. 1, no. 7, pp. 1104–1112, Jul. 2021, doi: 10.1021/acsestengg.1c00077.

[7] Y. Lv, J. Pan, T. Huo, Y. Zhao, and S. Liu, “Enhanced microbial metabolism in one stage partial nitritation-anammox system treating low strength wastewater by novel composite carrier,” *Water Res.*, vol. 163, p. 114872, Oct. 2019, doi: 10.1016/j.watres.2019.114872.

[8] M. Bahram, N. Mohseni, and M. Moghtader, *Emerging Concepts in Analysis and Applications of Hydrogels*. InTech, 2016. doi: 10.5772/61692.

[9] E. M. Ahmed, “Hydrogel: Preparation, characterization, and applications: A review,” *J. Adv. Res.*, vol. 6, no. 2, pp. 105–121, Mar. 2015, doi: 10.1016/j.jare.2013.07.006.

[10] G.-L. Zhu, Y.-Y. Hu, and Q.-R. Wang, “Nitrogen removal performance of anaerobic ammonia oxidation co-culture immobilized in different gel

carriers," *Water Science and Technology*, vol. 59, no. 12, pp. 2379–2386, Jun. 2009, doi: 10.2166/wst.2009.293.
[11] W. Song *et al.*, "The performance of co-immobilized strains isolated from activated sludge combined with Scenedesmus quadricauda to remove nutrients and organics in black odorous water," *Bioresour. Technol.*, vol. 345, p. 126571, Feb. 2022, doi: 10.1016/j.biortech.2021.126571.
[12] X. Lin, B. Li, M. Tian, X. Li, and J. Wang, "Denitrification effect and strengthening mechanism of SAD/A system at low temperature by gel-immobilization technology," *Science of The Total Environment*, vol. 900, p. 165599, Nov. 2023, doi: 10.1016/j.scitotenv.2023.165599.
[13] L. Hou *et al.*, "Advances in immobilized microbial technology and its application to wastewater treatment: A review," *Bioresour. Technol.*, vol. 413, p. 131518, Dec. 2024, doi: 10.1016/j.biortech.2024.131518.
[14] V. T. Tang *et al.*, "Immobilization of microorganisms in activated zeolite beads and alkaline pretreated straws for ammonium-nitrogen removal from urban river water," *Water Science and Technology*, vol. 85, no. 1, pp. 63–76, Jan. 2022, doi: 10.2166/wst.2021.496.
[15] S. Chero-Osorio, "Ion Exchange and Bioregeneration by Partial Nitritation/Anaerobic Ammonium Oxidation (Anammox) for Total Nitrogen Removal from Municipal Wastewater," University of South Florida, Tampa, FL, US, 2025. Accessed: Jan. 25, 2026. [Online]. Available: https://digitalcommons.usf.edu/
[16] A. A. van de Graaf, P. de Bruijn, L. A. Robertson, M. S. M. Jetten, and J. G. Kuenen, "Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor," *Microbiology (N Y).*, vol. 142, no. 8, pp. 2187–2196, Aug. 1996, doi: 10.1099/13500872-142-8-2187.
[17] V. Aponte-Morales, "Ammonium Removal from High Strength Wastewater Using a Hybrid Ion Exchange Biological Process," University of South Florida, Tampa, FL, US, 2015.
[18] S. Chero-Osorio, "Carbon Diversion, Partial Nitritation/Anammox Enrichment, and Ammonium Capture as Initial Stages for Mainstream Ion Exchange-Deammonification Process," University of South Florida, Tampa, FL, US, 2022.
[19] W. C. . Lipps, E. Burton. Braun-Howland, and T. E. . Baxter, *Standard methods for the examination of water and wastewater*. American Public Health Association, 2023.
[20] Y. Zheng, Y. Liu, and A. Wang, "Fast removal of ammonium ion using a hydrogel optimized with response surface methodology," *Chemical Engineering Journal*, vol. 171, no. 3, pp. 1201–1208, Jul. 2011, doi: 10.1016/j.cej.2011.05.026.
[21] M. He, T. C. A. Ng, S. Huang, B. Xu, and H. Y. Ng, "Ammonium removal and recovery from effluent of AnMBR treating real domestic wastewater using polymeric hydrogel," *Sep. Purif. Technol.*, vol. 296, p. 121376, Sep. 2022, doi: 10.1016/j.seppur.2022.121376.
[22] J. Wang, J. Liang, D. Ning, T. Zhang, and M. Wang, "A review of biomass immobilization in anammox and partial nitrification/anammox systems: Advances, issues, and future perspectives," *Science of The Total Environment*, vol. 821, p. 152792, May 2022, doi: 10.1016/j.scitotenv.2021.152792.
[23] M. Strous, J. J. Heijnen, J. G. Kuenen, and M. S. M. Jetten, "The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms," *Appl. Microbiol. Biotechnol.*, vol. 50, no. 5, pp. 589–596, 1998, doi: 10.1007/s002530051340.
[24] A. C. Anthonisen, R. C. Loehr, T. B. S. Prakasam, and E. G. Srinath, "Inhibition of Nitrification by Ammonia and Nitrous Acid," 1976.
[25] S. W. H. Van Hulle, H. J. P. Vandeweyer, B. D. Meesschaert, P. A. Vanrolleghem, P. Dejans, and A. Dumoulin, "Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams," Aug. 2010. doi: 10.1016/j.cej.2010.05.037.
[26] S. E. Vlaeminck, H. De Clippeleir, and W. Verstraete, "Microbial resource management of one-stage partial nitritation/anammox," *Microb. Biotechnol.*, vol. 5, no. 3, pp. 433–448, May 2012, doi: 10.1111/j.1751-7915.2012.00341.x.
[27] B.-J. Ni, M. Ruscalleda, and B. F. Smets, "Evaluation on the microbial interactions of anaerobic ammonium oxidizers and heterotrophs in Anammox biofilm," *Water Res.*, vol. 46, no. 15, pp. 4645–4652, Oct. 2012, doi: 10.1016/j.watres.2012.06.016.
[28] C. E. Lawson *et al.*, "Metabolic network analysis reveals microbial community interactions in anammox granules," *Nat. Commun.*, vol. 8, no. 1, p. 15416, May 2017, doi: 10.1038/ncomms15416.
[29] M. S. M. Jetten, L. van Niftrik, M. Strous, B. Kartal, J. T. Keltjens, and H. J. M. Op den Camp, "Biochemistry and molecular biology of anammox bacteria," *Crit. Rev. Biochem. Mol. Biol.*, vol. 44, no. 2–3, pp. 65–84, Jun. 2009, doi: 10.1080/10409230902722783.
[30] A. Malovanyy, J. Yang, J. Trela, and E. Plaza, "Combination of upflow anaerobic sludge blanket (UASB) reactor and partial nitritation/anammox moving bed biofilm reactor (MBBR) for municipal wastewater treatment," *Bioresour. Technol.*, vol. 180, pp. 144–153, Mar. 2015, doi: 10.1016/j.biortech.2014.12.101.
[31] N. Miladinovic and L. R. Weatherley, "Intensification of ammonia removal in a combined ion-exchange and nitrification column," *Chemical Engineering Journal*, vol. 135, no. 1–2, pp. 15–24, Jan. 2008, doi: 10.1016/j.cej.2007.02.030.
[32] Z. Song *et al.*, "Zeolite powder based polyurethane sponges as biocarriers in moving bed biofilm reactor for improving nitrogen removal of municipal wastewater," *Science of The Total Environment*, vol. 651, pp. 1078–1086, Feb. 2019, doi: 10.1016/j.scitotenv.2018.09.173.
[33] T. Kloknicer, A. Szabó, D. B. Sándor, and G. Filipcsei, "Challenges and Disadvantages of PVA-Based Media Application in Wastewater Treatment: A Mini-Review," *Environments*, vol. 12, no. 9, p. 294, Aug. 2025, doi: 10.3390/environments12090294.
[34] N. A. M. Zain, M. S. Suhaimi, and A. Idris, "Development and modification of PVA–alginate as a suitable immobilization matrix," *Process Biochemistry*, vol. 46, no. 11, pp. 2122–2129, Nov. 2011, doi: 10.1016/j.procbio.2011.08.010.