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Article

Performance Evaluation of a Pilot-Scale Aerobic Granular Sludge Integrated with Gravity-Driven Membrane System Treating Domestic Wastewater

Muhammad Ali,* Yogesh Singh, Luca Fortunato, Zahid Ur Rehman, Sarvajith Manjunath, Johannes S. Vrouwenvelder, Mario Pronk, Mark C. M. van Loosdrecht, and Pascal E. Saikaly*

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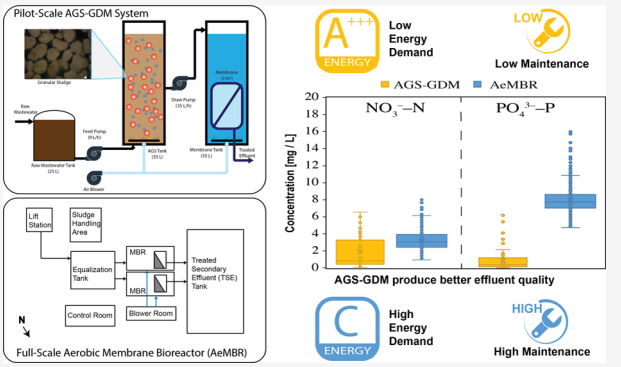
ABSTRACT: This study describes a novel integration of aerobic granular sludge (AGS) with a gravity-driven membrane (GDM) system at a pilot scale with a treatment capacity of approximately 150 L per day to treat raw domestic wastewater. The treatment performance and energy consumption of the AGS-GDM system were compared to the neighboring full-scale aerobic membrane bioreactor (AeMBR), treating the same wastewater at about 4000(±500) m^3 per day. The AGS-GDM system demonstrated superior nutrient (nitrogen and phosphorus) removal as compared to the AeMBR. The GDM unit was continuously supplied with AGS-treated effluent. The GDM unit started with high [>20 L per m^2 per h (LMH)] flux, which gradually declined. The flux remained quite stable after 15 days reaching 3 LMH after 35 days without any physical or chemical cleaning. Our results suggest that AGS-GDM is a viable technology for decentralized wastewater treatment and reuse in water-scarce regions. The AGS-GDM could easily replace conventional AeMBR technology in the wastewater treatment and reclamation market.



KEYWORDS: *aerobic granular sludge, gravity-driven membrane, decentralized wastewater treatment, water reuse*

1. INTRODUCTION

By 2030, the global demand for energy and fresh water is expected to increase by 40 and 50%, respectively.[1] To augment these depleting resources, municipal wastewater cannot be regarded anymore as merely a "waste" but as a valuable resource of water for reuse, energy, and materials/minerals.[2−4] Globally, more than 360 billion m^3 of wastewater is produced annually, out of which ∼50% is treated and only ∼10% is reused.[5,6] Currently, the most widely used biological wastewater treatment process is the conventional activated sludge (CAS) process, and it has been employed in most wastewater treatment plants (WWTPs) for over a century, where organic matter is aerobically converted to biomass and carbon dioxide.[7,8] In the CAS process, biological wastewater treatment is carried out by small microbial aggregates (size around 0.2 mm), also referred to as a floc or activated sludge, which typically grows in suspension in the treatment reactor. A subsequent settling tank (secondary clarifier) is used to separate activated sludge from the treated water by the sedimentation process. The main disadvantage of CAS technology is the separation of the slow-settling flocculent sludge from treated water. The settlers (also known as clarifiers) take up much more space than the bioreactors where the wastewater is treated. Besides, there are many operational challenges associated with these clarifiers such as sludge bulking and foaming, which deteriorate the treatment performance of CAS plants,[9,10] limitation to low mixed liquor suspended solid (MLSS) concentrations,[11] and the tendency to develop floating sludge.[12,13] Additionally, the CAS process is energy-intensive and usually requires 0.3−0.6 (typically 0.45) kWh per m^3 of wastewater treated,[14] where 50% of the energy consumed is attributed to aeration. It has been reported that about 3% of the annual electrical energy is consumed for wastewater treatment in the United States, resulting in the emissions of more than 45 million tons of greenhouse gases annually.[15] The ever-growing population increase and rapid urbanization sets an increased demand on land areas. Therefore, there is a desire to develop wastewater treatment technologies, which require less area and energy input and

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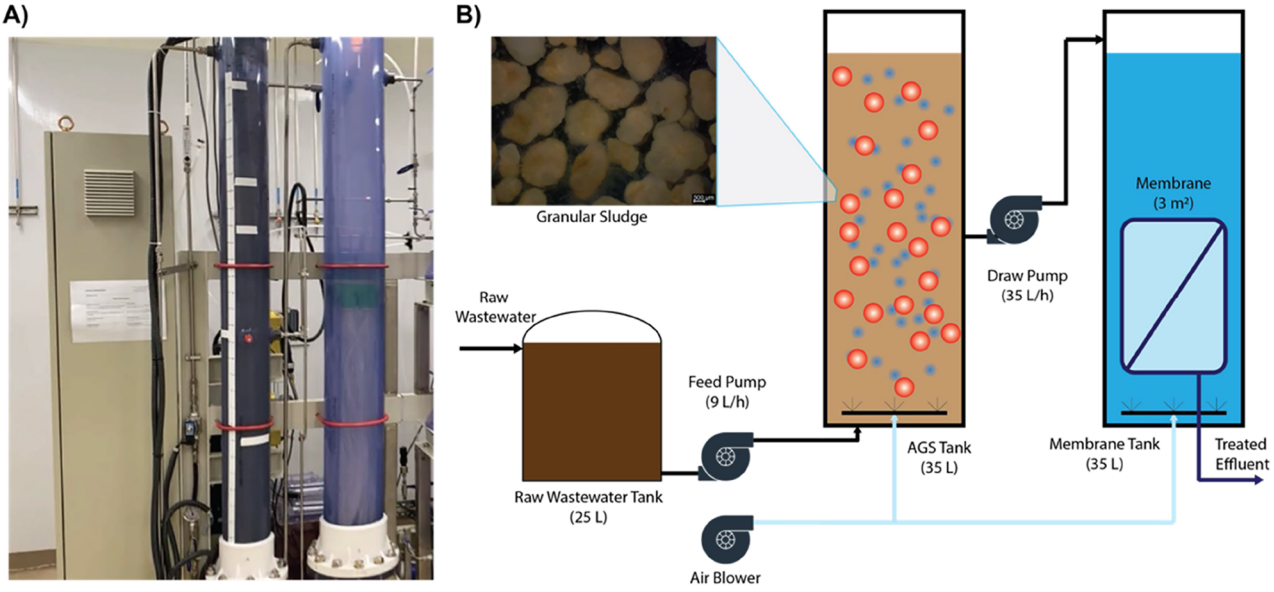

Figure 1. (A) Showing the actual photograph of the aerobic granular sludge gravity-driven membrane (AGS-GDM) system. (B) Schematic diagram of the AGS-GDM system. Fresh raw wastewater from the equalization tank of the KAUST WWTP was continuously filled into the raw wastewater tank using a wastewater line controlled with a float level switch installed in the tank.

should also have better operational stability and performance as compared to the CAS process.

Aerobic granular sludge (AGS) technology can outcompete the existing biological wastewater treatment technologies and holds great promise to become the standard for biological wastewater treatment in the future because of its small footprint, lower operational cost, effective simultaneous removal of carbon and nutrients (P & N) in a single reactor tank, and the ability to withstand toxic shock loading.[16,17] In AGS-based systems, combined carbon, nitrogen, and phosphorous removal occurs in a single tank based on a sequential batch process and relies on microbes (such as polyphosphate accumulating organisms shortly known as PAOs) that tend to grow in the larger size (>0.2 mm) granules rather than flocs.[17,18] Due to the high settling velocity of the granules, a separate settling tank is not required as in the CAS process, and as a result, only a single tank is needed for both biological treatment and settling.[19] Due to these characteristics, the AGS process has a 40−50% smaller footprint and 23% less electricity consumption than the CAS process.[14,16] The AGS technology was promptly scaled up from laboratory scale[20,21] to pilot scale[22,23] and then full scale in 2010 in Epe, the Netherlands.[24] Currently, there are 100 full-scale AGS installations worldwide, and the number is growing rapidly. Despite the abovementioned advantages, the effluent from AGS reactors still contains some suspended solids.[25] A higher-grade effluent is required to meet strict effluent quality or reuse standards, such as turbidity ≤2 (NTU), BOD ≤10 (mg/L), and no detectable *Escherichia coli* (*E. coli*; MPN/100 mL), which cannot be achieved with the CAS or AGS technology alone.[26,27] To address the water scarcity issue and achieve microbially safe effluent quality suitable for non-potable reuse, further polishing of the effluent from AGS would be required.[25,28]

To meet stringent wastewater treatment regulations, aerobic membrane bioreactor (AeMBR) technology using the CAS process is currently the gold standard for wastewater treatment and reuse. The AeMBR technology replaces the large clarifiers with a compact energy-driven membrane separation process.[29] However, the AeMBR process has important operational drawbacks such as membrane fouling, which requires frequent cleaning (chemical and physical) of the membrane, and is an energy-intensive process (typically requires >0.62 kWh per m^3) due to the energy-driven membrane filtration.[14] A recent study evaluated the electricity demand for various wastewater treatment technologies and concluded that a treatment process based on AGS has lower electricity requirements compared to (23%) activated sludge and (50−70%) well-optimized AeMBRs.[14] The high membrane operating cost and membrane fouling are still considered to be the biggest challenges in AeMBR technology.[30,31] Several efforts were made to integrate the AGS process with a submerged pressure-driven membrane filtration system;[32−35] however, the operational challenges related to biofouling and energy consumption remain unaddressed.

Gravity-driven membrane (GDM) processes operate at an ultra-low gravity pressure with less maintenance as compared to conventional membrane filtration systems such as in the AeMBR.[36] The GDM process is a passive membrane filtration process that is operated at subcritical flux, which does not cause extensive fouling and achieves membrane flux stabilization over time. Moreover, the GDM requires no energy input for filtration as the process is driven by the natural gravity pressure.[37] The GDM process has been tested for the treatment of different water streams including greywater, river water, and seawater pre-treatment for reverse osmosis (RO).[31,36−38] The GDM filtration is highly compatible with the AGS bioreactor due to its natural height (tubular shape design). The height of the AGS tank would provide a sufficient water pressure head to drive the GDM filtration process through gravity (without energy input). So far, there have been no studies on the integration of the AGS process with GDM filtration.

Therefore, the objective of this study was to develop a pilot-scale AGS-GDM system for domestic wastewater treatment and reuse and assess its performance, microbial community

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ACS EST Water XXXX, XXX, XXX−XXX

composition, and energy usage. Furthermore, the performance, microbial community composition, and energy usage of the pilot-scale AGS-GDM system were compared to a parallel-operated full-scale decentralized AeMBR, treating the same wastewater.

2. EXPERIMENTAL SECTION/METHODS

2.1. KAUST WWTP. The decentralized municipal WWTP at King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia (coordinate: 22°17′54.8″N39°07′07.7″E), has a treatment capacity of about 10,000 m^3/day. The average wastewater received at the treatment plant is around 4000 m^3/day. The treatment plant was designed as the AeMBR using a flat sheet Kubota membrane module (Kubota, Japan) containing microporous membranes made from polyolefin and having a nominal pore size of 0.4 μm. The layout of the AeMBR WWTP is shown in Figure S1. The treatment plant has a separate anoxic tank to facilitate the denitrification process to remove nitrogen from the wastewater. The treated effluent is stored in the treated effluent storage tank after disinfection through chlorination and later supplied to the nearby golf course for irrigation.

2.2. Design and Operation of the Pilot-Scale AGS-GDM System. The AGS-GDM system with a treatment capacity of about 100 L per day was established by integrating AGS with a GDM unit (Figure 1). A 35 L AGS reactor (height of 2000 mm and inner diameter of 152 mm) was designed to operate as a sequential batch reactor. The AGS reactor was seeded with granular biomass harvested from a full-scale Nereda (Nereda is a trademark owned by Royal HaskoningDHV for a proprietary AGS technology) installation located in Utrecht, the Netherlands. The cycle time was set at 4 h under steady-state conditions: 120 min for influent feeding, 90 min for reaction (aerobic and anoxic), 10 min for settling, and 20 min was allocated for effluent withdrawal. The volumetric exchange ratio was fixed at 50%, which corresponds to 8 h of hydraulic retention time. During the feeding phase, influent from the feed tank was gradually introduced from the bottom of the AGS reactor in a plug-flow mode using a variable speed magnetic gear pump (MG204XK, Ningbo Haoxin, China). Upon filling the AGS reactor to the desired level, the feeding mode was switched to the aeration mode. Air was supplied with a diaphragm membrane air blower (LP 40A, THOMAS YASUNAGA, Japan), and the airflow rate was controlled using a mass flow controller (Model 3660, Kofloc, Kyoto, Japan). An electric solenoid valve was used to effectively maintain the dissolved oxygen (DO) below the set point (2.5 mg/L) during the aeration phase (Figure 2). The DO was continuously monitored with an online DO Transmitter (DC-5110, Suntex, Taiwan). The pH was monitored in real time and controlled between the set points (7−8.5) with a pH controller (sc200, HACH, Germany). Acid or base pumps were turned on as needed to maintain the pH at the desired set points. Both pH and DO were measured in the middle of the reactor. Subsequently, the air blower was stopped to allow microbial granules to settle down at the bottom of the AGS reactor during the settling phase. After the settling phase, an effluent pump displaces the top half volume of the AGS reactor to the GDM tank.

A 50 L circular GDM tank was designed with dimensions: height of 1700 mm and diameter of 195 mm. The GDM tank was equipped with customized hollow fiber polyvinylidene fluoride microfiltration (nominal pore size 0.1 μm) membrane

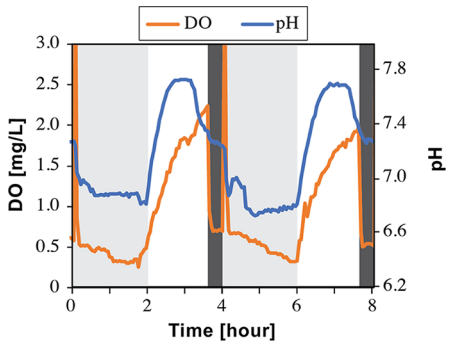

Figure 2. Typical profiles of DO and pH (both measured in the middle of the reactor) of the pilot AGS system during operation with real wastewater. The light gray-shaded region represents the feeding phase (influent flowrate was 8.75 L per h), the white-shaded region represents the reaction phase, and the dark, gray-shaded region represents the settling and withdrawal phases. The cycle time was set at 4 h: 120 min feeding, 90 min reaction (aerobic and anoxic), 10 min settling, and 20 min withdrawal.

module with a 3.5 m^2 effective membrane surface area (KOLON Industries, Inc., Korea). The height of the membrane module was 900 mm allowing a maximum of 800 mm water head above the membrane module. The membrane module was connected to a solenoid valve actuated with the level sensor placed within the membrane tank. The solenoid valve stops the filtration once the water level reaches 1000 mm height to keep the membrane wet, and it resumes filtration once the water level increases to 1500 mm height in the tank. The membrane tank was also connected with a diaphragm membrane air blower (LP 40A, THOMAS YASUNAGA, Japan) to facilitate air scouring of the membrane. During the chemical cleaning process, the membranes were soaked with a solution of sodium hypochlorite (0.1% w/v) as described elsewhere.[39]

During the start-up phase, the AGS reactor was operated with synthetic wastewater containing: 0.42 mM K_2HPO_4, 0.21 mM KH_2PO_4, 5 mM $NaC_2H_3O_2$, 0.8 mM $NaC_3H_5O_2$ (75−25% mixture of propionate to acetate), 3.5 mM NH_4Cl, 0.36 mM $MgSO_4$, 0.47 mM KCl, and 1 mL/L trace element solution according to ref 40. The operational parameters obtained during this period are summarized in Table 1. After start-up, the AGS reactor was operated for 1 year and was continuously fed with raw wastewater coming from the equalization tank of the KAUST WWTP. The average chemical oxygen demand (COD) and ammonium-nitrogen (NH_4^+-N) of the raw wastewater in the equalization tank were low in concentration, 194 ± 39 mgCOD/L and 18.5 ± 4.5 mgN/L (Table 2). This is lower than the typical concentrations of COD (450 mg/L) and NH_4^+-N (35 mg/L) in domestic wastewater.[11] To maintain COD and NH_4^+-N levels representative of domestic wastewater, the raw wastewater was supplemented with organic carbon (75−25% mixture of acetate-to-propionate) and nutrients. The current study focused on the direct comparison of electricity consumption between the aeration systems (blowers) of the pilot AGS-GDM unit and the full-scale AeMBR. To obtain the energy consumption data for the AeMBR, we sourced the information from the plant operator. In the case of the pilot AGS-GDM unit, the energy consumption was calculated by multiplying the power (W) of the blower with the time (h), the blower was in operation during the aeration phase.

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ACS EST Water XXXX, XXX, XXX−XXX

Table 1. Operational Parameters of the Pilot AGS-GDM System

description	value	unit	description	value	unit
AGS reactor diameter	0.152	m	MLSS concentration	9.2	kg/m^3
AGS reactor area	0.018	m^2	volumetric loading rate	2.67	$m^3/m^3/day$
AGS reactor height	1.950	m	sludge loading rate	0.13	kg COD/kg VSS/day
reactor volume	0.035	m^3	up flow velocity	0.49	m/h
feed time	120	min	feed flowrate	0.0088	m^3/h
reaction time	90	min	hydraulic retention time (HRT)	480	min
settle time	10	min	solid retention time (SRT)	17	day
draw time	20	min	anaerobic COD uptake	116.4	g COD/kg VSS/day
cycle time	240	min	anaerobic P release	49.7	g P/kg VSS/day
flowrate	0.1	m^3/day	$P_{release}/COD_{uptake}$	0.43	
influent COD concentration	0.450	kg/m^3	aerobic P uptake	55.8	g P/kg VSS/day
exchange ratio	0.50		N removal rate	14.6	g N/kg VSS/day

Table 2. Influent and Effluent Parameters of the KAUST AeMBR

		influent (equalization tank)			effluent
description	unit	minimum	maximum	average (±SD)	average (±SD)
TSS	mg/L	25	253	95.9 ± 24.6	1.2 ± 0.5
BOD_5	mg/L	50	260	126.1 ± 39.1	4.3 ± 1.2
COD	mg/L	94	452	194.3 ± 39.1	6.9 ± 2.4
NH_4^+-N	mg/L	5	25.3	18.5 ± 4.5	0
NO_3^--N	mg/L	1	3.8	2.1 ± 0.4	3.6 ± 1.3
PO_4^{3-}-P	mg/L	2.5	16.9	7.2 ± 2.4	7.8 ± 1.5
Flow	m^3/day	1310	6313	3919 ± 475	
total energy consumption	kWh	6269	11,865	8241 ± 742	
energy consumption by blowers	kWh	435	6010	3187 ± 615	
total energy consumption	kWh/m^3	1.6	4.8	2.1 ± 0.2	
energy consumption by blowers	kWh/m^3	0.1	1.3	0.81 ± 1.2	

2.3. Evaluation of GDM Performance. The membrane tank was operated under a head pressure supported by the water head above the membrane module. The average head pressure (1250 mmH_2O) is calculated considering the middle point of the membrane module. The permeate flow rate was measured in real time (every 5 min interval) using a flow meter (FTB324D, OMEGA, USA) installed on the permeate line. The permeate flux was measured using the following equation (eq 1):

$$J = \frac{Q}{A} \tag{1}$$

where J = permeate flux (L per m^2 per h, LMH); A = the effective membrane surface area of 3.5 m^2; Q = permeate flow rate (L/h), and the flow readings with maximum water head were considered for flux calculations.

In addition, the hydraulic resistance (m^{-1}, R) was calculated based on Darcy's law (eq 2):

$$R_{total} = \frac{TMP}{\mu_{20°C} \times J_{20°C}} \tag{2}$$

where TMP is the transmembrane pressure, which was the gravitational pressure supported by the water head during the filtration process, $\mu_{20°C}$ is the permeate viscosity at 20 °C, and $J_{20°C}$ is the flux measured in the experiment.

2.4. Measurement of Physical and Chemical Parameters. Influent and effluent samples were collected from the AGS-DGM system and filtered using a 0.45 μm pore size syringe disc filter (Jinteng, Tianjin, China). The concentrations of COD, phosphate (PO_4^{3-}), NH_4^+-N, nitrite-nitrogen (NO_2^--N), and nitrate-nitrogen (NO_3^--N) were measured using HACH kits (HACH, CO, USA) following manufacturer's instructions. The concentrations were measured by a spectrophotometer (D5000, HACH, CO, USA). The MLSS, total suspended solid (TSS), and colony-forming unit (CFU) in the samples were determined according to a modified Standard Methods for the Examination of Water and Wastewaters specifically for AGS (APHA, 2005). These measurements were conducted using triplicate samples obtained under stable flux conditions. A total of six (n = 6) samples were collected, three (n = 3) during the initial 35 days of flux measurement and the remaining (n = 3) during the subsequent 35 days of flux measurements after cleaning. The samples were collected during the quite stable flux phase from day 10 to day 35. Fresh samples were analyzed for *E. coli* by filtering them through a 0.22 μm pore size filter (Millipore). The filters were then placed on Petri dishes containing chromogenic agar (Sigma Aldrich) and incubated at 44 °C for 22−24 h. After incubation, the CFUs of total coliform and *E. coli* were counted. The sludge volume index (SVI) was measured by pouring 1000 mL of sample into a graduated measuring cylinder. The volume of the biomass was consequently recorded after 5 and 30 min. Only undiluted samples were used for SVI measurements.

2.5. Fluorescence In Situ Hybridization (FISH). Fresh biomass was harvested from the AGS reactor and homogenized using glass tissue grinders. Homogenized biomass was fixed with 4% (w/v) paraformaldehyde and stored at 4 °C for 12 h. The biomass was pasted on Teflon-coated glass slides. The FITC-labeled EUB mix probe composed of equimolar EUB338, EUB338II, and EUB338III was used to stain most members of Eubacteria,[41] and the Cy3-labeled PAOmix probe (composed of equimolar PAO462, PAO651, and PAO846 probes) was used to hybridized *Candidatus* Accumulibacter clade I and II simultaneously.[42] The samples were observed with a LSM700 confocal laser-scanning microscope equipped with diode lasers (488, 555, and 639 nm) (Carl Zeiss, Oberkochen, Germany).

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2.6. DNA Extraction, 16S rRNA Gene Sequencing, and Microbial Community Analysis. Fresh biomass was harvested from the AGS reactor under steady-state conditions. DNA extraction of sludge samples was done using a FastDNA Spin kit for soil (MoBio Laboratories, Inc., Carlsbad, CA, USA) with a slightly modified version of the standard protocol as described elsewhere.[43] DNA concentration was measured using a Qubit dsDNA HS/BR assay kit (Thermo Fisher Scientific, USA). Amplicon libraries for the bacteria/archaea 16S rRNA gene variable region 4 (abV4C) were prepared by a custom protocol based on an Illumina protocol as described previously.[17,44] The purified sequencing libraries were pooled in equimolar concentrations and diluted to 2 nM. The samples were paired-end sequenced (2 × 300 bp) on a MiSeq (Illumina, USA). Forward and reverse reads were trimmed for quality using Trimmomatic v. 0.32 with the settings SLIDINGWINDOW:5:3 and MINLEN: 225.[45] The trimmed forward and reverse reads were merged using FLASH v. 1.2.7 with the settings m 10 M 250.[46] The trimmed reads were dereplicated and formatted for use in the UPARSE workflow.[47] Taxonomy was assigned using the UCLUST classifier as implemented in the assign_taxonomy.py script in QIIME,[48] using the MiDAS database v.4.0.[49] All bioinformatic processing was done via RStudio IDE (2022.2.3.492) running R version 4.2.1 (20220623) and using the R packages: ampvis (2.7.27).[50]

3. RESULTS AND DISCUSSION

3.1. Reactor Start-Up and Performance. Initially, the pilot AGS-GDM system was operated with synthetic wastewater for a month after seeding with granular sludge to optimize reactor operation and conversion efficiencies. Following successful optimization with synthetic wastewater, the AGS-GDM system was operated continuously for 1 year with real wastewater. As previously mentioned, the raw wastewater from the equalization tank was supplemented with organics and nutrients to make the concentration of COD (450 mg/L) and NH_4^+-N (35 mg/L) more representative of domestic wastewater. The SVI decreased significantly during this period and the difference between SVI_5 (40−50 mL/g) and SVI_{30} (35−45 mL/g) decreased during the operation. The percentage of granular (>0.2 mm) sludge was >80% and barely any granules larger than 0.2 mm were detected in the AGS-treated effluent going into the GDM tank. The concentration of TSS in the wastewater influent was found to be 174.5 ± 6.4 mg/L, while the TSS concentration in the AGS-treated effluent was measured at 68 ± 2.2 mg/L. Similarly, the influent contained high levels of total coliforms and *E. coli*, ranging from 23−73 × 10^4 CFUs/100 mL and 13−42 × 10^4 CFUs/100 mL, respectively. In contrast, the AGS-treated effluent showed significant reductions in bacterial levels, with total coliforms ranging from 30 to 33 CFUs/100 mL and *E. coli* ranging from 2 to 4 CFUs/100 mL. These results demonstrate the effectiveness of AGS treatment in removing TSS and reducing bacterial contamination in wastewater.

The AGS-GDM system achieved >90% removal of nutrients, and the treated effluent had very low residual concentrations of NH_4^+-N (0.6 ± 2.0 mg/L), NO_2^--N (0.3 ± 0.5 mg/L), NO_3^--N (0.7 ± 0.4 mg/L), and PO_4^{3-}-P (0.8 ± 1.3 mg/L) (Figure 3). The effluent quality of the AGS-GDM system was superior to the neighboring full-scale AeMBR, which contains higher concentrations of NO_3^--N (5.6 ± 3 mg/L) and PO_4^{3-}-P (7.8 ± 1.5 mg/L) in the effluent. The AGS-GDM unit produced an effluent that has a slightly higher residual COD (32.7 ± 10.1 mg/L) as compared to the AeMBR effluent (6.6 ± 2.1 mg/L).

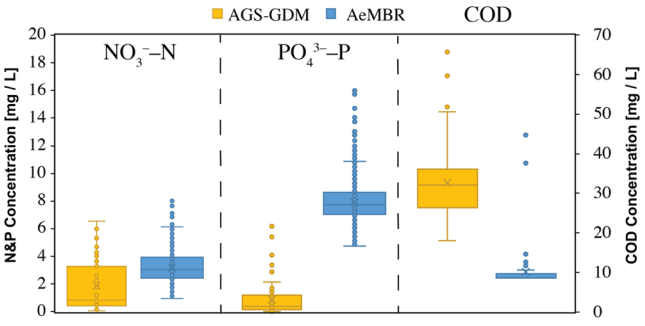

Figure 3. Treated effluent water quality from the AGS-GDM and AeMBR systems. Ammonium and nitrite concentrations in the effluent were below 1 mg N/L during the 1 year operation with real wastewater. Influent COD, NH_4^+, and PO_4^{3-} were 450 mg, 35 mg N, and 20 mg P per L, respectively. Nitrite and nitrate concentrations in the influent were below 1 mg N/L during this period. The box plot displays the five-number summary including minimum, first quartile (25th percentile), median, third quartile (75th percentile), and maximum value. The points below the minimum and above the maximum values are outliers.

In the AeMBR or CAS process, nitrogen removal efficiency primarily depends on the nitrification and denitrification capacity of the microbial community.[11] The denitrification process in the AeMBR or CAS is highly dependent on the recycle flow from the oxic (nitrification) to the anoxic tank and sometimes requires the supply of external carbon. External carbon dosage is costly and can lead to elevated BOD concentrations in the effluent when overdosed.[51] One of the main advantages of the AGS process is that the biological nitrogen removal processes are not separated in different oxic and anoxic tanks. Aerobic granules have a layered structure that provides niche subenvironments for the co-existence of various functionally important microbial groups, facilitating nitrifying microbes in the outer aerobic layer, denitrification and phosphorous removal in the inner anoxic/anaerobic layers of granules.[17,52,53] This layered structure of granules enables the simultaneous removal of organics and nutrients (N & P) from the wastewater.[18]

In AGS, the influent was pumped into the reactor from the bottom with an upward velocity (0.5 m/h) without mixing or aeration. Under these anaerobic feeding conditions, easy biodegradable organic carbon (e.g., acetate or propionate) will be converted into storage polymers such as polyhydroxyalkanoates or glycogen by PAOs or glycogen accumulating organisms (GAOs), respectively.[54] GAO and PAO compete with each other for organic carbon substrates in the feed stage, but GAO does not contribute to P removal.[8] The average anaerobic COD uptake during the feeding phase was calculated as 116.4 ± 31.3 g COD/kg VSS/day (Table 1). The uptake of the biodegradable substrate was accompanied by the release of orthophosphate into the bulk liquid as can be seen from the relatively high phosphate concentration (86.8 ± 33.8 mg/L) at the beginning of the aeration period (Figure 4). An average anaerobic orthophosphate release into the bulk liquid during the feeding phase was calculated as 49.7 ± 13.0 g P/kg VSS/day. The ratio between orthophosphate release and COD uptake was estimated at around 0.43 (Table 1), which is typical of AGS or enhanced biological phosphorous removal systems enriched with PAOs and showing good P removal.

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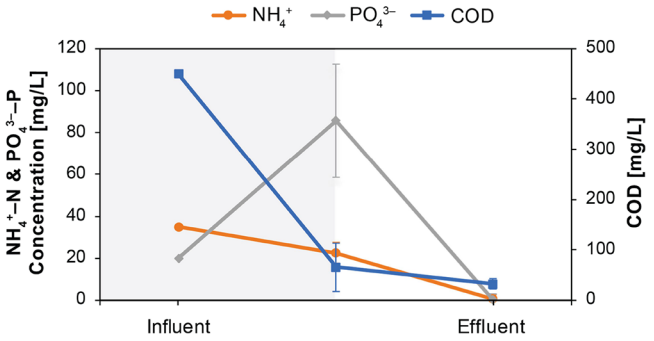

Figure 4. Profile of COD, NH_4^+, and PO_4^{3-} concentrations within a cycle. Nitrite and nitrate concentrations in the effluent were below 1 mg N/L. Nitrite and nitrate concentrations in the influent were below 1 mg N/L. The gray-shaded zone represents the anaerobic feeding phase, and the white-shaded zone represents the aeration phase.

Taken together, these results support the role of PAOs in phosphorous removal in the AGS-GDM unit. This was further supported by microbial community data in Section 3.2.

After the feeding period, the reactor was aerated and thus mixed, while the DO concentration was maintained below 2.5 mg/L. At the start of the aeration period, NH_4^+ and PO_4^{3-} concentrations peaked due to the mixing of treated water in the top with influent in the lower part of the reactor. The concentrations after mixing, combined with the amount of wastewater fed, can be used as an indication of the loading rate of the cycle but need to be corrected for phosphorus release and NH_4^+ adsorption. NH_4^+ and PO_4^{3-} removal occurred during the 90 min aeration phase (Figure 4). The orthophosphate in the bulk solution was converted into intracellular polyphosphate by PAOs during aeration at an average rate of 55.8 ± 15.0 g P/kg VSS/day (Table 1), which corresponds to the net phosphorus removal in the AGS reactor. NH_4^+ was mainly removed in the AGS system through simultaneous nitrification and denitrification at an average removal rate of 14.6 ± 3.4 g N/kg VSS/day (Table 1). Simultaneous nitrification and denitrification can be facilitated by maintaining the DO at the desired set point.[55] The DO value was maintained below the set point (3 mg/L) to allow for simultaneous nitrification and denitrification in the granular biomass with denitrification mainly occurring in anoxic volume inside the granules[56] where oxygen cannot penetrate.

3.2. Microbial Community Composition. A total of 18 samples from the AGS-GDM system and AeMBR were sequenced yielding between 30,909 and 64,895 nonchimeric and quality-filtered reads after bioinformatic processing (Table S1). The nonchimeric, quality-filtered reads were clustered into 1797 OTUs at 97% identity. A heatmap distribution of the top 25 taxa classified down to the genus level or the lowest classifiable taxonomic level was plotted to visualize the variation of individual taxa in AGS (flocs, granules, and MLSS) and the AeMBR (MLSS) (Figure 5). *Candidatus* Accumulibacter, a known PAO, was present in higher relative read abundance in the AGS biomass and was particularly present in higher relative read abundance in granules (30%) than flocs (16%) (Figure 5). FISH performed on crushed homogenized granules showed a high abundance (~40%) of the PAO population (Figure 6), confirming sequencing data. PAO relative read abundance was lower (~5%) in the AeMBR (MLSS). The higher abundance of PAOs agreed with the P removal performance in the AGS-GDM system. A GAO

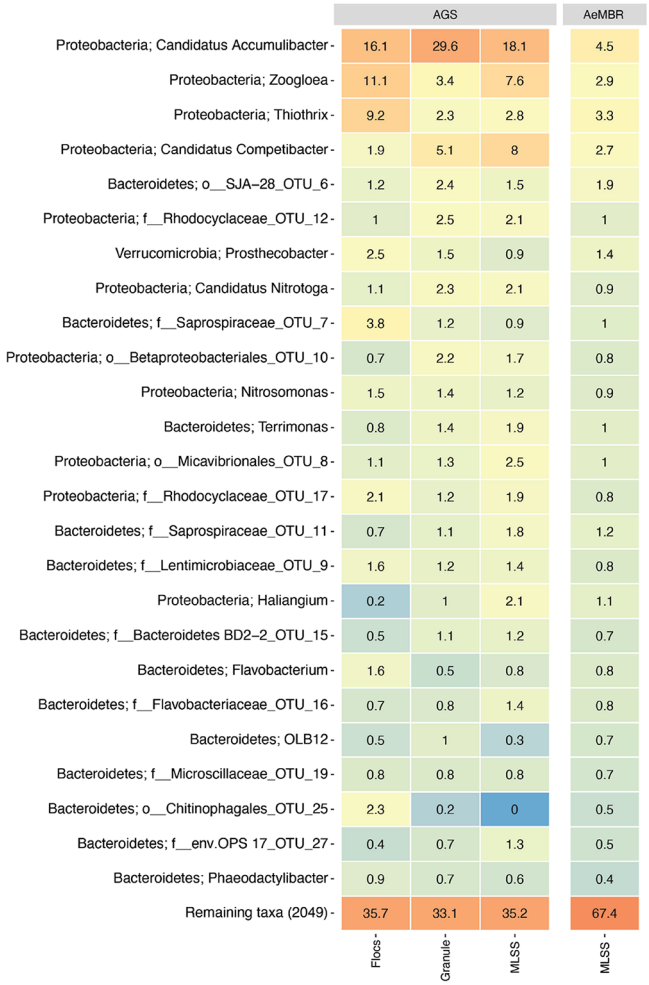

Figure 5. Heatmap distribution of the top 25 taxa classified down to the genus level or the lowest classifiable taxonomic level (f and o represent family and order, respectively). MLSS corresponds to the mixed liquor-suspended solids in AGS and the AeMBR.

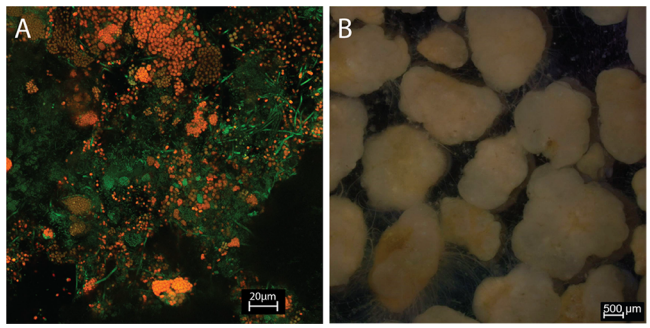

Figure 6. (A) Confocal laser scanning microscope images of the granular biomass from the AGS-GDM reactor. Fluorescence in situ hybridization was performed with a FITC-labeled EUB mix probe composed of equimolar EUB338I, EUB338II, and EUB338III (green) for most members of Eubacteria and a CY3-labeled PAO mix probe composed of equimolar PAO462, PAO651, and PAO846 (red) for *Ca.* Accumulibacter, which is a known polyphosphate-accumulating organism (PAO). The image shows the distribution of PAOs (orange) and all bacteria (green) in a granule. (B) Showing a photographic image of the granular biomass. Scale bars represent 20 and 500 μm for Panels A and B, respectively.

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ACS EST Water XXXX, XXX, XXX−XXX

population (*Candidatus* Competibacter) was also present in the AGS reactor but at a lower relative abundance than PAOs. The genus *Thiothrix* had a higher relative read abundance in flocs compared to granules. *Thiothrix* is characterized as filamentous bacteria.[57] *Thiothrix* was also detected (3%) in the AeMBR system. Nitrifiers (*Nitrosomonas* and *Ca.* Nitrotoga) were also detected in high abundance (>1%) in the AGS system, and their abundances in the AeMBR were less than 1%.

3.3. GDM Performance Evaluation and Flux Stabilization. Before the start of the actual GDM operation, the virgin membrane resistance was measured ($9.93 \pm 0.11 \times 10^{-11}$ m^{-1}) with clean water under constant gravitational pressure (1.25 m) supported by the water head (Table S2). The permeate flow rate at maximum water head pressure was used to calculate the membrane flux. After testing with clean water, the GDM unit was then operated using AGS-treated effluent. The GDM unit started with high flux (>20 LMH) that gradually declined to reach 3 LMH after 35 days without any cleaning (Figure 7). Afterward, the membrane was then cleaned with

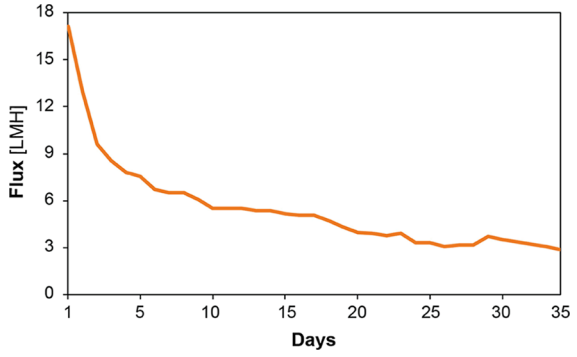

Figure 7. Variations of specific flux over 35 days before cleaning with air scouring. Flux presented as liter per m^2 membrane surface area per hour (LMH).

only air scouring, and 81% of the flux was recovered (Table S2). The GDM was constantly operated with AGS-treated water for another 35 days without any cleaning and a similar trend in flux was observed. The membrane was hereafter chemically cleaned and 91% of the flux was recovered (Table S2). The trend in fouling over time characteristic of the GDM was due to operation at low flux. These results highlight the long and passive operation of the GDM system without the need for frequent cleaning. The GDM permeate demonstrated exceptionally low turbidity, measuring at 0.15 ± 0.02 NTU, and both TSS and *E. coli* were below the detection limit. These results highlight the exceptional quality of GDM permeate.

It is pertinent to mention that the current study did not specifically investigate the separate removal efficiencies of organic carbon and nutrients in AGS and GDM tanks, as this was beyond the scope of the study. However, we acknowledge that such an investigation is needed in future studies to evaluate the removal performance of each unit separately.

3.4. Energy and Chemical Usage of the Full-Scale AeMBR. The total energy consumption of the full-scale AeMBR was 2.1 ± 0.2 kWh/m^3 with 0.81 ± 1.2 kWh/m^3 only for aeration blowers (Table 2). The energy consumption for the complete treatment process also includes the energy for the administrative building, air conditioning, streetlights, and other allied infrastructure. Therefore, we only compared the electricity consumption of the aeration systems (blowers) of the pilot AGS-GDM unit and the full-scale AeMBR. The energy consumption of the pilot (0.35 kWh m^{-3}) AGS-GDM unit is comparable with the previously reported full-scale (0.2 kWh m^{-3}) AGS plant.[16] The AGS process consumes less energy because of the absence of mixers, conventional recycle pumps, settlers, and sludge return pumps.[14] Overall, the previous study on full-scale AGS installation reported about 50% less energy consumption as compared to CAS (0.4 kWh m^{-3}) plants.[16] In summary, AGS-GDM systems produced better effluent quality as compared to CAS and the AeMBR even with less input of energy.

The AeMBR uses an energy-driven membrane filtration system, operated at high constant flux to increase the output of the system. The AeMBR operation at high flux leads to early accumulation of membrane foulants, consequently, clogging the membrane.[58] The removal of foulants causing membrane clogging requires expensive chemical cleaning. The AeMBR consumes large quantities of NaOH (32 ± 3 mL/m^3 of wastewater treated) and NaOCl (11.6 ± 12.6 mL/m^3 of wastewater treated) for membrane cleaning (Table 3). The accumulation of membrane foulants intensifies the frequency of physical and chemical cleanings (Malaeb et al.,[59] Vanysacker et al.[60]), and the replacement of membrane modules becomes inevitable in the event of irreversible fouling (Le-clech et al.[61]). Flocculant sludge in the AeMBR produces filamentous microbial aggregate and soluble extracellular polymeric

Table 3. Actual Chemical Consumption and Membrane Flux Data of the Full-Scale AeMBR Plant

	caustic soda (NaOH)		sodium hypochlorite (NaOCl)			
month	[L/month]	[mL/m^3]	[L/month]	[mL/m^3]	wastewater treated [m^3/month]	membrane flux [LMH]
Jan	3620	33.7	360	3.4	107,329	14.6
Feb	3632	33.2	2100	19.2	109,252	14.8
Mar	3922	32.7	900	7.5	120,045	16.3
Apr	3660	33.3	360	3.3	109,826	14.9
May	3720	31.9	392	3.4	116,645	15.8
Jun	4700	38.3	630	5.1	122,802	16.7
Jul	3860	30.8	4400	35.1	125,503	17.0
Aug	3720	29.1	1500	11.7	128,024	17.4
Sep	3600	26.1	300	2.2	137,875	18.7
Oct	3720	30.4	4570	37.3	122,447	16.6
Nov	3600	30.8	900	7.7	117,019	15.9
Dec	3720	35.7	100	1.0	104,292	14.1
average	3790 ± 303	32 ± 3	1376 ± 1558	11 ± 13	118,422 ± 9754	16 ± 1

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ACS EST Water XXXX, XXX, XXX−XXX

substances, which further contribute to clogging the membrane. In contrast, GDM operates at low (subcritical) flux, which leads to less frequent clogging of the membrane and consequently less chemical cleaning and irreversible clogging.[38] However, attaining low productivity in terms of flux would necessitate an increase in membrane area, increasing the capital cost.[38] Therefore, it is crucial for future studies to determine the optimal reactor heights to achieve higher water productivity. Such studies will yield comprehensive insights into the implications and limitations of GDM operation, specifically in terms of its impact on water production and area footprint.

4. CONCLUSIONS

In this study, a pilot-scale AGS-GDM (capacity of about 0.1 m^3 per day) unit was developed and successfully operated in an arid climate to treat real wastewater and its performance was compared to a neighboring full-scale AeMBR, treating the same wastewater. The following conclusions are drawn:

1. The AGS-GDM unit demonstrated better nutrient (N & P) removal and produced superior quality effluent as compared to the neighboring AeMBR. The AGS-GDM unit could achieve high nutrient removal rates due to the presence of PAOs in the AGS tank and simultaneous nitrification and denitrification due to a controlled DO set point.
2. The GDM unit, operated under constant gravitational pressure supported by the water head (1.25 m), showed a consistent flux of around 3 LMH head until 35 days without any need for cleaning while filtering AGS-treated water.
3. The aeration system of the AGS-GDM unit required less (0.35 kWh m^{-3}) energy as compared to the aeration system of a fully functional AeMBR (0.8 kWh m^{-3}).

These results demonstrate that the AGS-GDM technology could be a promising viable alternative to the AeMBR for decentralized wastewater treatment and reuse in water-scarce regions. The study demonstrated that good quality effluent can be achieved using this novel (AGS-GDM) technology with less input of energy as compared to the neighboring full-scale AeMBR. Only *E. coli* was measured in this study, and future work should focus on characterizing the log removal performance of viruses across the operational ranges of the GDM and comparing it with MBRs.

■ ASSOCIATED CONTENT

Data Availability Statement

Raw sequencing data were deposited at the National Center for Biotechnology Information (NCBI) under accession number PRJNA892278.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.3c00178.

The layout of full-scale MBR plant; sequencing statistics; and membrane fouling characteristics (PDF)

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Notes

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