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Effect of powdered activated carbon (PAC) addition and nitrogen mixing during anaerobic fill period on aerobic granular sludge (AGS) sequencing batch reactor (SBR) process performances

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Abstract. This study investigated the effects of powdered activated carbon (PAC) addition and feeding strategy on aerobic granular sludge (AGS) formation and organic micropollutant (OMP) removal. Three sequencing batch reactors were operated: PAC-AGS1 (PAC addition and static fill without mixing), PAC-AGS2 (PAC addition and anaerobic mixing during fill period), and a control AGS reactor (anaerobic mixing during fill period). Results demonstrated that PAC significantly accelerated granulation and improved sludge settleability during early operation period. PAC-AGS1 exhibited the fastest decrease in SVI30 (25 mL/g) and achieved a granular fraction of approximately 90% by day 49, indicating rapid formation of compact and stable granules. Meanwhile, anaerobic fill with nitrogen purging slightly delayed early-stage granulation in PAC-AGS2. All systems achieved effective COD removal; however, PAC-AGS1 showed enhanced OMP removal efficiency, probably due to the combined effects of adsorption and biodegradation within stable granular structures. Microbial community analysis revealed distinct shifts at the family level, with PAC-AGS1 enriching structure-supporting bacteria. The results indicated that PAC addition under stable aerobic feeding conditions promotes rapid granule formation, structural stability, and improved micropollutant removal. This study highlights the significance of both PAC as an adsorptive nucleus and static feeding strategy to enhance AGS performance for advanced wastewater treatment applications.

Keywords: aerobic granular sludge; biodegradability; organic micropollutants; powdered active carbon; sequencing bath reactor

1. Introduction

The presence of organic micropollutants (OMPs) in aquatic environments has emerged as a critical environmental issue due to their persistence, bioaccumulation potential, and adverse impacts on ecosystems and human health [1]. OMPs include a diverse range of substances such as pharmaceuticals, personal care products, pesticides, and industrial chemicals, many of which are only partially removed by conventional wastewater treatment processes [2, 3]. Their continuous discharge, even at trace concentrations, can lead to chronic toxicity in aquatic organisms, disruption of endocrine systems, and the development of antimicrobial resistance [4]. Moreover, the complex chemical structures and varied physicochemical properties of OMPs pose significant challenges to

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effective monitoring and treatment [5].

The increasing attention towards sustainable and efficient wastewater treatment technologies has led to the exploration of innovative methods that can address the complexities associated with the removal of OMPs and the enhancement of treatment efficacy. The increasing discharge of OMPs such as pharmaceuticals, personal care products, and industrial chemicals into aquatic environments has raised significant concerns regarding their persistence, toxicity, and potential for bioaccumulation [6, 7]. Conventional wastewater treatment processes are often inadequate for the complete removal of these trace contaminants, necessitating the development of advanced treatment technologies.

Various technologies have been explored for the removal of organic micropollutants, including adsorption (e.g., activated carbon), advanced oxidation processes (ozonation, UV/H_2O_2), and membrane filtration (nanofiltration, reverse osmosis) [8-10]. While these methods achieve high removal efficiency, they are often energy-intensive and costly. Compared with physico-chemical methods, biological treatment of micropollutants has gained increasing attention because of its advantages such as low cost, high removal efficiency and simple process design [11]. In recent years, aerobic granular sludge (AGS) has emerged as a promising alternative to conventional treatment due to its compact structure, high biomass retention, high settling velocity and ability to create simultaneous aerobic, anoxic, and anaerobic zones within granules, enabling enhanced biodegradation of various pollutants such as carbon, nitrogen and phosphorus [12-14]. However, despite these benefits, the stable formation and long-term maintenance of granular sludge remain challenging, particularly under fluctuating operational conditions and high-strength wastewaters [15]. However, the removal efficiency of OMPs in biological processes can be limited, necessitating supplementary treatment [16].

Powdered activated carbon (PAC) is widely recognized for its surface area and high adsorption capacity for various organic contaminants and has been extensively used in wastewater treatment for micropollutant removal [17, 18]. Recent studies have demonstrated that PAC can also play a synergistic role in enhancing AGS formation [19]. When integrated into AGS systems, PAC can serve multiple functions directly adsorbing micropollutants, provide additional surface area for microbial attachment, and potentially influencing the formation, stability and characteristics of granules [20]. Moreover, PAC can act as a carrier for microbial communities, enhance mass transfer within granules, and improve the adsorption and subsequent biodegradation of micropollutants [21]. Despite its potential, the mechanisms underlying PAC-assisted AGS formation and its contribution to OMP degradation are not yet fully understood.

This study aimed to investigate the effects of PAC addition and anaerobic mixing during the feeding period on AGS formation in sequencing batch reactor (SBR) and its performance in removing OMPs. Three SBRs were operated by adding PAC to two of them (PAC-AGS1 and PAC-AGS2) and the rest one is a baseline reactor (AGS) without PAC. Nitrogen gas purging during anaerobic fill period was implemented to the AGS and one of PAC-AGS (PAC-AGS2) to assess the impact of these two factors on granule formation and pollutant removal. The morphology and properties of granular sludge in OMPs treatment with three reactors were compared. The dynamic changes of microbial community structure and the distribution characteristics in two reactors were studied. Furthermore, aerobic microbial biodegradability of OMPs was evaluated using three different AGSs obtained from each reactor through a real-time respirometric bioassay.

2. Materials and methods

2.1 AGS reactor setup and operation conditions

Three 2.7L-aclylic SBRs were set up as AGS bioreactor in the lab. Each reactor was equipped with an up-flow airlift type of aeration system for enhancing AGS formation by providing sufficient shear force and stable fluid circulation flow for recirculation. A thin cylindrical air-driven pipe was installed from the top to the bottom at the center of the reactor, with a diffuser connected to the air-driven pipe placed at the bottom. The working volume of each reactor 2.4 L, and actual reactor's height to diameter (H/D) ratio was 10. The SBRs were operated with volumetric exchange rate (VER) of 46%~60% under room temperature conditions.

Three SBRs were operated in parallel for 85 days by feeding a synthetic wastewater by blending secondary wastewater from 'O' WWTP and glycerin-based organic carbon source. Coal-based PAC (DUKSAN Pure Chemical Co. Ltd, South Korea) was initially added to SBRs (PAC-AGS1 and PAC-AGS2) at 500 mg/L. The PAC was characterized by a specific surface area of 956.3 m2/g and an average pore size of 1.874 nm [22]. In addition to two PAC-added SBRs, control SBR (AGS) was operated. Different from PAC-AGS1, one of PAC-added SBRs (PAC-AGS2) and AGS were operated by employing nitrogen gas purged mixing at 1 L/min during anaerobic fill period.

Operating cycle mode for all the SBRs were identical by alternating cycles of anaerobic fill, aeration and settling to promote granulation. The reactor operated 8 cycles per day (identical 6 hr HRT), and each3h cycle consisted of anaerobic fill (30 min), aeration (135 min), settling (12 min) and withdrawal (3 min). The air flow rate was set at 0.3 mL/min, which is identical to the superficial air velocity (SAV) at 1.1 cm/s. The influent was introduced through the bottom port using a peristaltic pump (BT100F, Lead Fluid Technology Co., Ltd., China) during fill period. An Arduino system (Arduino Uno (R3), Arduino. cc, Italy) automatically controlled the SBR operation for feeding, reaction, and withdrawing.

2.2 Seeds and influent characteristics

The reactors were inoculated with activated sludge obtained from a domestic WWTP ('S' city, Korea). The initial concentrations of mixed liquid suspended solids (MLSS) in three reactors were identically set at 3000 mg/L. The secondary effluent was obtained from a AGS pilot plant in 'O' WWTP in Chungcheongbuk-do, Korea, and was used as the influent after blending synthetic carbon source. Glycerin based-external carbon source (SC-60) and nitrate were separately added to the influent to set the COD/N ratio at 5.0 which is known as an optimal one for AGS formation [23]. The chemical oxygen demand (COD) concentration and organic loading rate (OLR) of the influent during operating period were 172.7 $\pm$ 44.8 mg/L and 0.70 $\pm$0.16, respectively. The concentration of total nitrogen was 33.0 $\pm$ 6.1 mg/L. Thus, the COD/N ratio varied in the range of 5.3 $\pm$ 1.1, which was close to the designed value (COD/N =5).

2.3 Analytical methods

2.3.1 Granule sludge and water characterization

Operating parameters include mixed liquor suspended solids (MLSS) concentration, sludge volume index (SVI_{30}), and granule composition which were regularly monitored to assess AGS formation. Also, COD and TN concentration in both the influent and effluent were regularly measured to evaluate the biological removal performances. COD, TN, MLSS and SVI were measured according to standard methods [24]. Optical microscopy was employed to examine the morphology and structural integrity of the granules obtained from the SBRs.

2.3.2 The analysis of microbial community

To investigate the structural changes in microbial community, polymerase chain reaction (PCR) amplification was applied to the mixed liquor samples collected from all reactors after 85 days of operation. After homogenizing the sludge samples, 250 mg of solid sample and 300 µL liquid sample was collected, and the resulting pellet was used for DNA extraction. Total genomic DNA was extracted by using the QUIAGEN DNeasy® PowerSoil® Kit (QUIAGEN, Germany). 1st PCR conditions were performed with an initial heat at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 5 min. The universal primer pair with Illumina adapter overhang sequences used for the first amplification was as follows: the 16S rRNA gene amplicon PCR forward primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAAGACAGCCTACGGGNGGC WGCAG-3') and the reverse primer (5'-CTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGG GTATCTAATCC-3'). 2nd PCR used the same thermal cycling conditions as the 1st PCR, with the exception that only 10 amplification cycles are applied. Real time qPCR assays were performed on the CFX96 Real-Time PCR Detection System (BioRad, C1000, USA) using the primer sets and analyzed with KAPA SYBR FAST Universal 2X qPCR Master KAPA Biosystems (Illumina Inc., USA). The final purified product is then quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platform) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). And then we sequenced using the MiSeq platform (Illumina, San Diego, USA). These PCR products were used for pyrosequencing analysis which was conducted by the Roche/454 GC Junior system (Macrogen, Seoul, Korea).

2.3.3 Respirometric bioassay for estimating biodegradation potential of OMPs

To estimate the biodegradability of PAC-AGS and AGS in the presence of OMPs, an aerobic bioassay was conducted using a real-time BOD monitoring system (BODTrak II, Hach, USA). The apparatus is characterized by continuously measuring changes in oxygen partial pressure resulting from aerobic respiration inside a sealed BOD bottle and providing real-time BOD values at the fixed time intervals of 15 min. The working volume of each bottle was 355 mL, and all samples were prepared in duplicate for both AGS inocula (PAC-AGS1 and AGS). The bottles were immediately sealed and loaded on the instrument.

Biotic control (inoculum only) and positive control (acetate only added as a carbon source) were also tested in parallel. Acetate and OMPs were added at 50 mg COD/L and 1 mg/L, respectively, to satisfy the BOD measurement range between 0 and 70 mg/L. The added OMPs were atenolol (ATL) and isoproturon (IPT), all of which are the commonly detected micropollutants in wastewater and treated effluents [25]. Two different inoculum of PAC-AGS1 and AGS (100 mg/L), obtained from the reactor, were each added separately to the BOD bottles. Detailed experimental conditions for different bioassay sets are summarized in Table 1. To each bottle, 5 mL of nitrification inhibitor solution (0.16 g/L, Hach Formula 2533) and one BOD nutrient buffer pillow (Hach, USA) were added. Potassium hydroxide powder was placed in the headspace compartment of the bottle to absorb CO_2 generated during aerobic respiration. The experiment was carried out in a temperature-controlled incubator maintained at 25 °C for 5 days.

Table 1. Experimental design of batch respirometric assays. Inoculum type (PAC-AGS or AGS), OMPs (ATL, IPT), and biodegradable substrate (acetate) are shown

Sample	Inoculum	Added OMP	Carbon source
Biotic control	PAC-AGS or AGS		-
ATL only	PAC-AGS or AGS	ATL	-
Ac+ATL	PAC-AGS or AGS	ATL	Acetate
Biotic control	PAC-AGS or AGS	-	-
IPT only	PAC-AGS or AGS	IPT	-
Ac+IPT	PAC-AGS or AGS	IPT	Acetate

Once the BOD results are obtained, the cumulative BOD curves were analyzed using the three-parameter Gompertz model as shown in Eq. (1) [26].

$$C(t) = C_{max} \times \exp\left[-\exp\left[\frac{R_m \times e^{(\lambda - t)}}{C_{max}} + 1\right]\right] \tag{1}$$

where, C(t) =BOD consumption at time t (mg/L)

C_{max} =Extent of BOD consumption (mg/L).

R_m =Rate of BOD consumption (mg/L/d).

λ =lag time (d).

3. Results and discussion

3.1 Performances of SBRs: MLSS, SVI, and effluent quality

MLSS concentrations in three reactors for 85 days of operation are shown in Fig.1. At the start of operation, MLSS concentrations were set at 3000 mg/L. MLSS concentration varied during operation as granulation proceeded (Fig. 1). In addition to the granulation state, the organic loading rate (OLR) affected the MLSS concentration. The secondary effluent from the pilot-scale AGS process was used as the influent of which quality was suddenly deteriorated due to operational problems in the pilot plant. Based on MLSS variation, the operating period could be divided into three phases: the initial granulation period (phase I, days 1–35), the granulation inhibition period (phase II, days 36–49), and the granulation recovery period (phase III, days 50–85). At the start of operation (phase I), the addition of PAC rapidly increased MLSS concentration up to 4,990 mg/L in the AGS reactors (PAC-AGS1, PAC-AGS2) compared to the AGS reactor without PAC addition. Lower OLRs (below 0.4 kg COD m^{-3} d^{-1}) during phase II adversely affected the AGS reactor by severely decreasing MLSS concentrations below 2,000 mg/L within 30 days. Abrupt famine conditions may have led to the disintegration of granular biomass structure in all reactors regardless of PAC addition [27]. Perhaps mixing during anaerobic fill period may have also affected MLSS concentration: PAC-AGS1 with static fill could retain slightly higher MLSS concentration than the others during this phase. The operational problem figured out in the pilot plant and the OLR restored to the same level as in phase I (0.8 kg COD m^{-3} d^{-1}) in phase III. As the phase III started, the MLSS concentration in all three reactors began to rise and reached approximately 4,300 mg L^{-1} after day 71 (Fig. 1). Except for phase II, the OLR was maintained between 0.6 and 1.1 kg COD m^{-3} d^{-1} which was considered highly suitable for initiating and

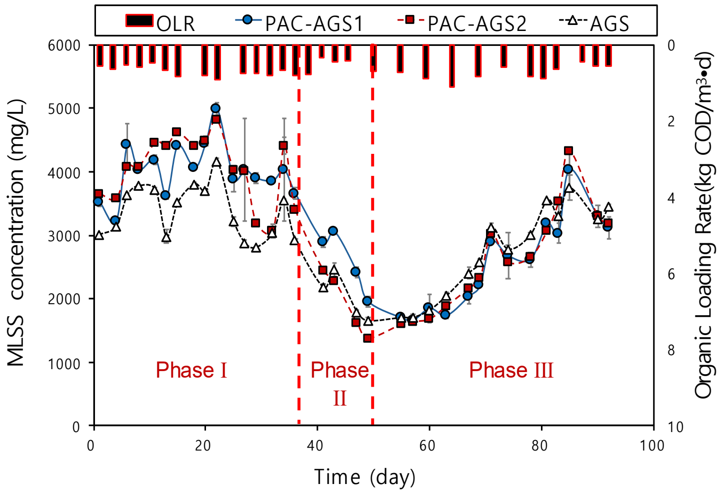


Figure 1. Variation of MLSS concentration as a function of time in three AGS reactors (PAC-AGS1, PAC-AGS2, and AGS reactors)

developing granular sludge in the early stage. For these phases, the F/M ratio varied from 0.2 to 0.55 kg COD kg^{-1} MLSS d^{-1}, indicating that both OLR and F/M conditions are important for successful AGS and PAC-AGS formation. Compared to AGS, PAC addition was significantly effective by playing a crucial role as a nucleus for accelerating AGS formation and reducing biomass loss during the startup of AGS reactor operation [28].

Influent and effluent COD concentrations during the operation period are shown in Fig. 2. Throughout phase I, influent COD fluctuated between approximately 130 and 230 mg/L, while effluent COD concentration in all reactors remained below 30 mg/L, resulting in COD removal efficiency ranging between 82 and 98% in all reactors. The effects of both PAC addition and the mixing strategy during the fill period were insignificant for COD removal. During phase II, when OLR abruptly decreased, effluent COD was maintained and even somewhat decreased below 15 mg/L. The effects of influent perturbation on effluent COD were significant in phase III rather than phase II. Effluent COD concentration increased up to 50 mg/L during the first 14 days in all reactors, and then decreased to below 40 mg/L after day 67, indicating that treatment performance was recovered.

Fig. 2(b) shows the variation of total nitrogen (TN) concentrations in the influent and effluent. During phase I, effluent TN concentrations gradually increased in all reactors, ranging from approximately 25 to 35 mg/L. In phase II, a pronounced decrease in TN concentration was observed, reaching minimum values of approximately 8-12 mg/L probably due to reduced influent nitrogen concentrations. As the influent composition returned to that of phase I during phase III, effluent TN increased moderately with increasing influent TN. Overall, nitrogen removal was not significant in any of AGS reactors, with effluent TN concentration of approximately 15-25 mg/L. Unexpectedly, anaerobic fill mixing did not improve TN removal performance probably due to insufficient COD available for denitrification. The COD/N was maintained at approximately 5.8 ± 2.0 throughout the operational period, which is close to the optimal value for AGS formation but insufficient for efficient biological TN removal [29]. Therefore, this COD/N ratio was adopted because the primary objective of this study was AGS formation rather than nitrogen removal.

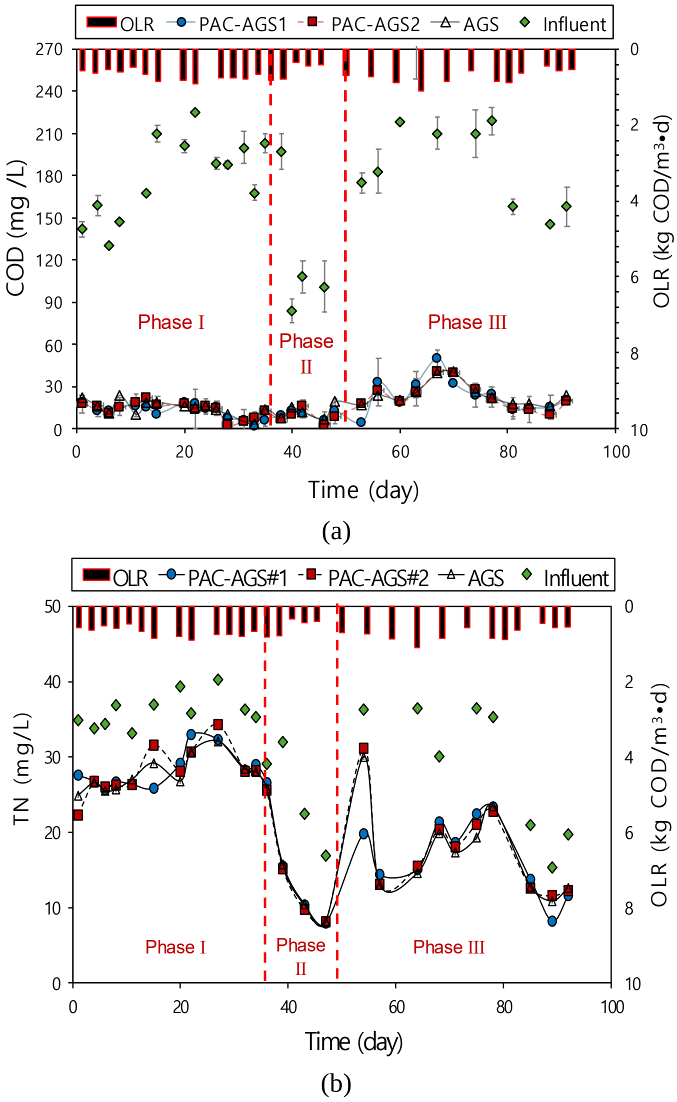
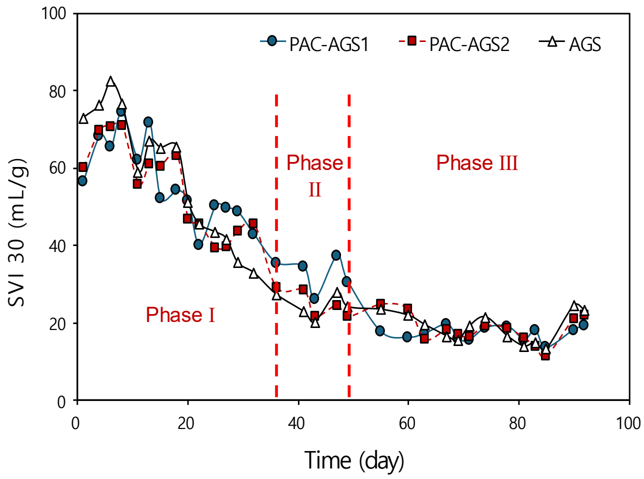


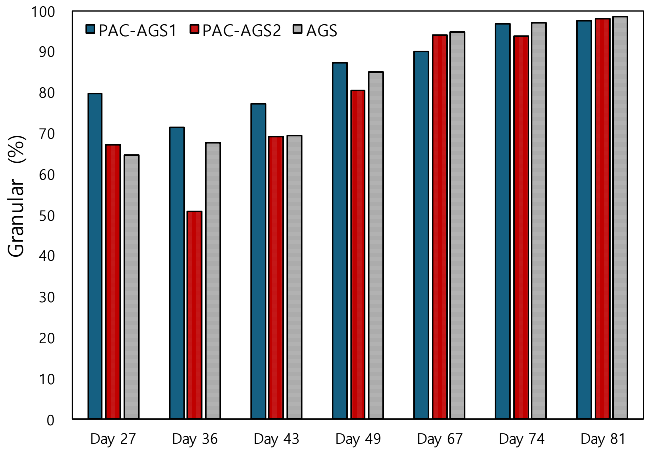
Figure 2. Variations of COD and total nitrogen (TN) concentrations in influent and effluent of three AGS reactors during operation period

3.2 Formation and characteristics of granular sludge

The development of sludge settleability and granulation behavior was systematically evaluated in three AGS reactors under different operational conditions. Fig. 3(a) shows the temporal variations of SVI_{30} in the three AGS reactors. At the startup period, the SVI_{30} value in AGS was approximately 80 mL/g which is within a satisfied range for sludge settling in CAS. However, SVI_{30} values of two PAC-added AGS reactors were below 60 mL/g indicating the dominance of flocculent sludge and the initial sludge of granule formation due to PAC addition. The granular sludge fraction was defined as the proportion of solids with a particle size ≥200 μm relative to the



(a)



(b)

Figure 3. (a) Variation of SVI_{30} in three AGS reactors during operation period and (b) evolution of granular sludge fraction in PAC-AGS1, PAC-AGS2, and AGS reactors during operation

total solids mass in the reactor. On day 27, PAC-AGS1 exhibited the highest granular fraction (~80%), followed by PAC-AGS2 and AGS (Fig. 3(b)). SVI_{30} tended to gradually decrease below 40~50 mL/g after 20 days in all reactors. From day 27, the SVI_{30} of AGS became lower than those of PAC-added bioreactors, although the reason is unclear. Abrupt decrease in OLR in phase II did not severely influence the SVI_{30} compared to MLSS. This indicated that famine condition caused biomass washout, but granulated sludge can endure this harsh condition by maintaining good settleability. AGS likely contained a higher proportion of well-settling flocculent biomass or compact micro-flocs, which can lower SVI without contributing to true granule formation [30]. In contrast, PAC-AGS maintained a higher granular percentage despite a slightly higher SVI, reflecting the stabilizing role of PAC in promoting and sustaining mature, dense granules rather than relying on floc compaction alone.

As the OLR was restored in phase III, a steady decrease in SVI_{30} was observed in all AGS reactors regardless of PAC addition and anaerobic fill mixing. During phase III, stable SVI_{30} values below 25 mL/g were maintained and SVI_{30}/SVI_5 ratio was consistently maintained above 0.9, indicating that granular sludge with very high settling velocity was dominant. It is well established that when granulation stabilizes, granular sludge is formed, settleability improves, and the SVI_{30}/SVI_5 ratio approaches 1 [31]. The granular fraction in PAC-AGS1 increased more rapidly than in the other reactors, reaching approximately 90% by day 49. Contrary to our expectation, PAC-AGS2, which employed nitrogen gas mixing during the anaerobic filling phase, did not exhibit enhanced nitrogen removal efficiency compared to PAC-AGS1. PAC-AGS1 maintained slightly higher MLVSS concentrations than PAC-AGS2 during phase I and II, indicating improved biomass retention and granule development. Granulation performance in PAC-AGS1 occurred more rapidly and to a greater extent than in PAC-AGS2. This difference might be attributed to variations in substrate distribution during the anaerobic fill period. In PAC-AGS1, static feeding allowed substrates to accumulate around the sludge bed, thereby promoting microbial attachment and granule growth. In contrast, nitrogen gas mixing in PAC-AGS2 resulted in a more uniform substrate distribution, reducing this localized effect [32]. All reactors achieved high granule fractions (>95%), with slightly higher and more stable values observed from Phase III onward. Although PAC addition contributed to higher MLSS concentrations and lower SVI values during the initial operational period, its effects became negligible in Phase III after the systems had stabilized.

The morphological evolution of sludge flocs or granules in the three reactors throughout the operation period is shown in Fig. 4. At the start of operation (day 1), the sludges were observed to be the typical activated sludge flocs, however those in the PAC-added AGS reactors (PAC-AGS1 and PAC-AGS2) appeared denser and agglomerated around the PAC nucleus. During Phase I, the initial operation phase, granulation progressed rapidly in all three reactors. By day 20, distinct granular sludge was observed in all reactors; however, PAC-AGS1 and PAC-AGS2 developed larger and more compact aggregates compared to the control AGS. In particular, PAC-AGS1 showed smoother surfaces and more spherical shapes, indicating that more rapid and stable granulation can be achieved under this condition [19]. The presence of PAC appeared to promote microbial attachment and aggregation, serving as a structural nucleus for granule growth. Thereby, granulation was particularly steady and pronounced in the two PAC-amended reactors (PAC-AGS1 and PAC-AGS2). However, during phase II (days 43–49), as the organic loading rate (OLR) decreased, the morphology of the granular sludge changed from predominantly circular aggregates to a more heterogeneous three-dimensional structure. Small sludge floc fragments were also observed alongside the granular sludge (Fig. 4). Such morphological destabilization under reduced OLR has been previously reported, as insufficient substrate supply can weaken extracellular polymeric substance (EPS) production and the structural integrity of granules [33].

During phase III, when the OLR returned to the normal range, the granular sludge in all three reactors reverted to a predominantly circular morphology, and small scattered sludge flocs gradually reorganized into granular structures. Over long-term operation, the granule size progressively increased, which is consistent with the observed very low SVI values. By day 76, PAC-AGS1 had already developed large, compact and structurally stable granules, whereas those in PAC-AGS2 and AGS appeared less uniform and more porous. The improved compactness in PAC-amended systems can be attributed to the role of PAC as a ballast material that promotes microbial aggregation and enhances mechanical strength [34]. Overall, the results demonstrate that PAC addition, particularly under static feeding without mixing during fill period, significantly enhanced granule

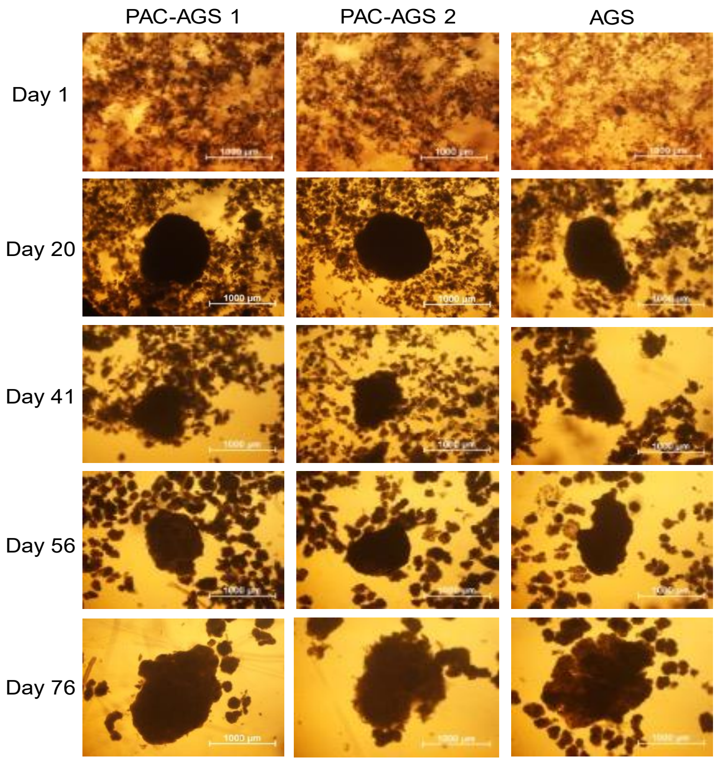


Figure 4. Microscopic images showing the development of aerobic granules in PAC-AGS1, PAC-AGS2, and AGS reactor during operation period

formation, compactness, and long-term structural stability. Even after 80 days of operation, PAC particles were observed attached to the surfaces of the granules, confirming that both AGS systems with PAC addition remained stable during long-term operation.

3.3 Microbial community structure analysis

Microbial community structure analysis was conducted to identify the key microbial populations in AGS granules formed under different conditions. The microbial community structures of PAC-AGS1, PAC-AGS2, and AGS were analyzed at the family level (Fig. 5). At the family level, the microbial consortia of PAC-AGS1, PAC-AGS2, and AGS were also comparable, although subtle differences were evident in the relative abundance of certain taxa. The communities were dominated by *Nakamurellaceae*, *Chitinophagaceae*, *Nitrospiraceae*, *Verrucomicrobiaceae* and *Aurantibacillaceae*, with several other families such as *Comamonadaceae*, *Zoogloeaceae* and *Sphaerotilaceae* present at lower abundances. All these groups are recognized as key microbial contributors to AGS formation. In particular, *Nakamurellaceae,* a family belonging to the phylum *Actinomycetota*, is known to play a role in the formation of sludge granules and structural stabilization in aerobic granular sludge systems [35, 36]. These bacteria contribute to the overall structure and functionality of the granules, thereby impacting wastewater treatment processes. PAC-AGS1 exhibited the highest relative abundance of *Nakamurellaceae*, suggesting the enhancement of glycogen-accumulating organisms, which are commonly associated with stable granule structure

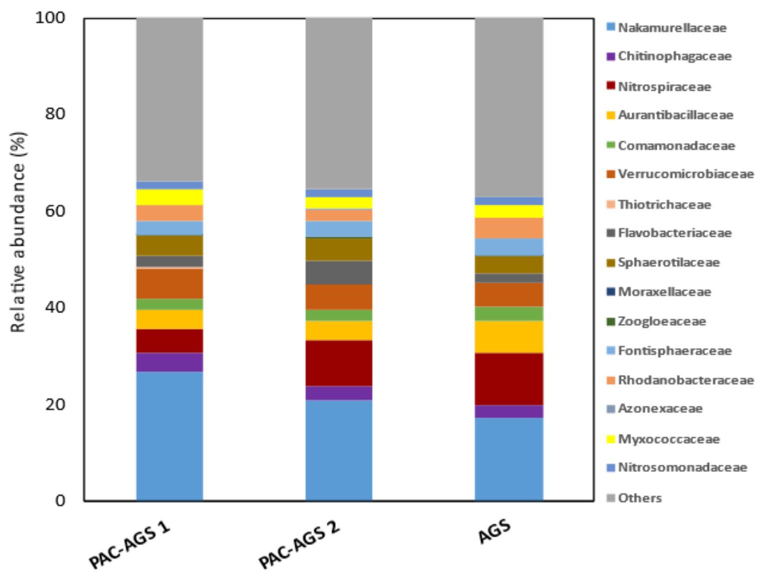
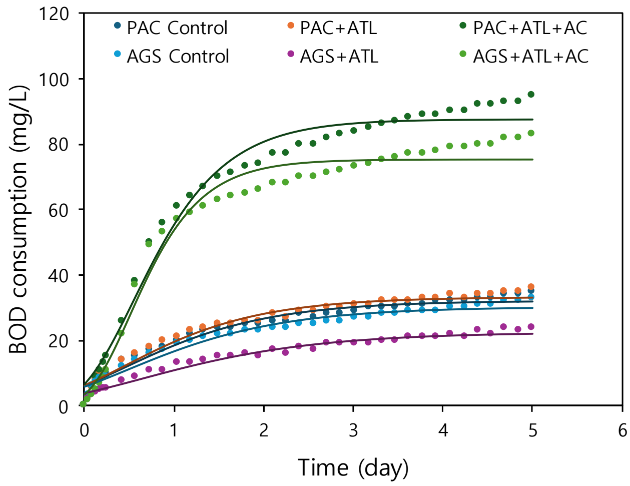


Figure 5. Microbial community structural differences in AGSs taken on day 85 from three AGS reactors at the family level

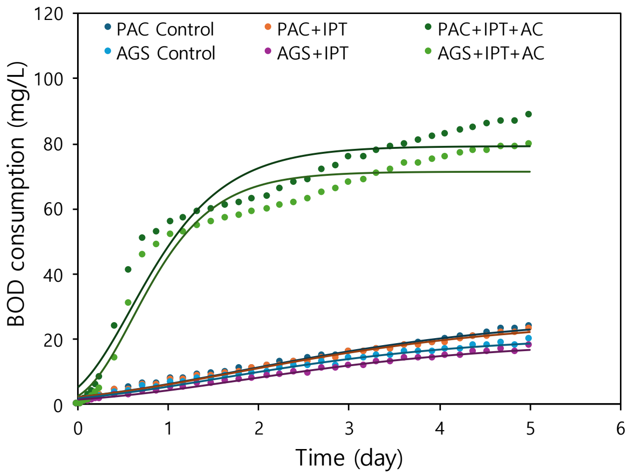
and carbon storage under feast-famine conditions. In contrast, the abundance of *Nakamurellaceae* decreased in PAC-AGS2 and further in AGS, indicating the nitrogen gas purging may have partially altered carbon competition dynamics during granulation. Nitrospira-related families (e.g., *Nitrospiraceae*) were more enriched in PAC-AGS2 and AGS compared with PAC-AGS1, implying relatively stronger nitrifying activity under intermittent nitrogen purging conditions. Meanwhile, families associated with denitrification and heterotrophic metabolism, such as *Comamonadaceae*, *Rhodanobacteraceae*, and *Zoogloeaceae*, affiliated with the phylum *Pseudomonadota*, were consistently detected across all reactors but displayed moderate variations in abundance [37]. *Aurantibacillaceae*, *Chitinophagaceae*, and *Flavobacteriaceae*, affiliated with the phylum *Bacteroidota*, are frequently associated with aerobic granular sludge systems. Members of these families contribute to granule formation and structure through the production of extracellular polymeric substance (EPS), which increases cell hydrophobicity and strengthens intercellular adhesion, thereby facilitating aggregation [38, 39]. They were present in all reactors, although their relative abundance was slightly lower in PAC-AGS1. In the PAC-added AGS reactors, PAC likely supplemented structural support and adsorbed organics, reducing the selective pressure for high EPS-producing populations like *Aurantibacillaceae*. Overall, the results indicate that while the microbial community structures of PAC-AGS and AGS systems are largely stable and robust, the addition of PAC as a nucleus may induce slight shifts at the family level, potentially influencing functional performance like granulation.

3.4 Bioassay for evaluating OMP biodegradability by AGS

A respirometric bioassay was conducted to evaluate the biodegradability of OMPs using AGS taken from two reactors (PAC-AGS1 and AGS) via real-time microbial oxygen consumption. Both ATL and IPT are frequently detected in municipal wastewater, however they exhibit distinctly



(a)



(b)

Figure 6. The effects of OMPs of (a) ATL and (b) IPT on BOD consumption by PAC-AGS 2 and AGS. The curve depicts the Gompertz model fitting of the real-time BOD consumption data.

different physicochemical characteristics. ATL is hydrophilic pharmaceutical compound, whereas IPT is a relatively hydrophobic herbicide that can exert inhibitory effects on microbial activity. These two chemical compounds were selected as representative OMPs for this bioassay due to their contrasting properties. Microbial oxygen consumption was expressed in terms of BOD consumption for 5 days and the results are presented in Fig. 6. Across most tested conditions, including the biotic control, OMP-only, and acetate with OMP (Ac+OMP), PAC-AGS consistently exhibited higher BOD consumption than AGS, irrespective of OMP type. Upon OMP addition, the BOD respiration in AGS decreased relative to those of the corresponding controls (AGS control), suggesting inhibition by OMPs to AGS biomass. In contrast, PAC-AGS maintained relatively

Table 2. Gompertz parameters obtained by fitting the model to BOD consumption curve estimating the effects of three OMPs of ATL and IPT on granules taken from PAC-AGS2 and AGS reactor.

OMP	Sample	AGS				PAC-AGS2			
		C_{max} (mg/L)	R_m (mg/L/d)	λ (d)	R^2	C_{max} (mg/L)	R_m (mg/L/d)	λ (d)	R^2
Atenolol	Biotic control	30.42	11.33	-0.03	0.96	32.27	12.99	-0.45	0.96
	ATL only	22.59	7.41	-0.47	0.97	33.46	14.22	-0.42	0.97
	Ac+ATL	75.39	61.30	0.05	0.98	87.67	55.83	-0.03	0.98
Isoproturon	Biotic control	21.35	4.58	-0.19	0.99	27.62	5.19	-0.20	0.98
	IPT only	19.81	4.09	-0.02	0.99	26.38	5.12	-0.15	0.99
	Ac+IPT	71.56	52.29	0.09	0.93	79.23	49.88	-0.01	0.95

higher activity, indicating reduced susceptibility to OMP-induced inhibition. When an easily biodegradable carbon source (acetate, 50 mg/L) was co-added with OMP, both AGS and PAC-AGS showed rapid and substantial increases in BOD consumption. Notably, PAC-AGS exhibited a greater increase in BOD consumption than AGS, indicating that consortium of PAC-AGS could degrade further the OMP as well as organic matters due to enhanced microbial activity without inhibition.

While these observations provide qualitative insights, the application of the Gompertz model enables a more quantitative evaluation of substrate utilization kinetics. The model was applied to BOD consumption data for 5 days, and the three parameters of the extent of BOD consumption (C_{max}), maximum BOD consumption rate (Rm), and lag period (λ) are summarized in Table 2. As described in Eq. (1), Cmax represents the maximum cumulative BOD consumption, reflecting the overall biodegradation potential of the microbial consortium. Rm corresponds to the maximum BOD consumption rate, indicating microbial metabolic activity, whereas λ represents the lag phase required for microbial adaptation before active biodegradation begins. Therefore, higher Cmax and Rm values together with shorter lag periods can be interpreted as indicators of enhanced microbial activity and biodegradation performance.

Comparison of the control systems clearly showed that microbial activity in PAC-AGS was higher than that in AGS alone. This enhanced activity is likely attributed to the presence of slowly biodegradable substrates adsorbed onto PAC, which can be gradually utilized by microorganisms as a sustained carbon source [19]. This interpretation is also supported by the microbial community analysis, where PAC-AGS exhibited favorable populations associated with carbon storage and granule stability, suggesting enhanced metabolic capacity.

Upon OMP addition (ATL and IPT), the Cmax values in AGS (22.59 and 19.81 mg/L, respectively) were lower than those of the corresponding controls (30.42 and 21.35 mg/L, respectively), indicating limited biodegradation or inhibitory effect on microbial activity [40]. Consistently, AGS exhibited a reduced BOD profile, whereas PAC-AGS maintained comparable or slightly higher activity, suggesting that PAC alleviated inhibitory effects, likely due to preventing the direct exposure of OMP to microorganisms by adsorption and allowing slow OMP degradation. With acetate co-addition, both systems showed similarly rapid increase in BOD consumption during the first two days, followed by a plateau. The Cmax values of AGS in the presence of acetate with ATL and IPT were 75.39 and 71.56 mg/L, respectively, whereas higher values were observed in PAC-AGS (79.23 and 74.00 mg/L, respectively), supporting the enhanced resistance of PAC-AGS to OMP-induced inhibition. This improvement is likely attributable to adsorption of OMPs onto PAC, which mitigated inhibitory effects and sustained microbial activity, thereby enhancing organic matter utilization under co-metabolic conditions. Overall, the results confirm that PAC addition strengthened granular sludge stability, mitigated inhibitory effects of OMPs, and enhanced organic matter removal, particularly under co-metabolic conditions [41].

4. Conclusions

This study demonstrated that the addition of PAC significantly enhances the performance of AGS in treating wastewater containing inhibitory OMPs. PAC improved granule formation by playing a dual role of nucleus and micro-carriers, resulting in more compact, denser, and structurally stable granules with better settleability and higher biomass retention. Microbial community analysis revealed that PAC increased the dominant taxa toward groups associated with granulation and OMP tolerance. These changes contributed to the improved resilience of PAC-AGS when exposed to toxic OMPs. Respirometric assays further confirmed the PAC mitigated OMP-induced inhibition, enabling higher biodegradation activity and maintaining metabolic stability. Overall, the findings highlight the dual role of PAC in AGS systems: accelerating granule development and strengthening structural integrity while simultaneously improving biological performance and microbial robustness under OMP stress. This integration may not only improve pollutant elimination but also promote the development of robust and mature granular sludge, making wastewater treatment more efficient, sustainable, and adaptable to emerging contaminants.

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