

Comparative Evaluation of Activated Sludge, Biogrow Biofilm, and Hybrid Systems for the Removal of Pollutants from Shrimp Pond Wastewater

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Abstract

Litopenaeus vannamei shrimp farming is a segment of aquaculture with significant economic potential. However, the wastewater produced during this process contains various pollutants, including salinity, phosphate, hydrogen sulfide, ammonia, nitrate, and nitrite. If it is discharged untreated, these pollutants can contribute to environmental pollution. This study aimed to compare the effectiveness of activated sludge, Biogrow, and a combination of both systems in reducing pollutant concentrations in shrimp pond wastewater. The experiment was conducted over 8 days in a batch system using Greenbio 1103 bacterial resin. Physical and chemical characteristics of the wastewater were analyzed on days 0, 2, 4, 6, and 8. Data were analyzed using Two-Way ANOVA, followed by Tukey's *post-hoc* test. The results indicated a significant interaction ($p < 0.05$) between treatment duration and treatment media in reducing ammonia, COD, phosphate, and TSS concentrations. Among the systems tested, the Biogrow medium exhibited the highest treatment efficiency, achieving removal rates of 99.5% for ammonia (from 15.18 to 0.079 mg/L), 57.1% for COD (from 1402.9 to 601.7 mg/L), 93.8% for phosphate (from 1.27 to 0.079 mg/L), and 42.9% for TSS (from 980 to 560 mg/L). In comparison, activated sludge alone achieved lower removal rates: 87.2% for ammonia, 45.4% for COD, 91.9% for phosphate, and 24.5% for TSS. The enhanced performance of Biogrow was attributed to its large specific surface area, which facilitated bacterial attachment and supported the development of a stable biofilm structure.

Keywords

Activated Sludge, Biogrow, Combination, Shrimp Pond, Wastewater Treatment

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1. INTRODUCTION

A pond is an artificial aquatic ecosystem designed to cultivate various aquatic organisms, including marine and brackish water species. One aquaculture activity with strong potential to support the local economy is farming vannamei shrimp (*Litopenaeus vannamei*) (Aktur and Yeumine, 2020). In Central Java, particularly in the coastal area of Kendal, several locations are used for vannamei shrimp farming, including the Y Shrimp Pond, located within a rice field. This shrimp pond covers about 2 hectares and includes several active ponds and two wastewater retention ponds. However, the pond is not equipped with a Wastewater Treatment Plant (WWTP). As a result, pond drainage water settles in a retention pond for a period before being discharged directly into the environment.

Wastewater from shrimp ponds can contain various pollutants that may harm the environment at high concentrations or when they exceed established quality standards. Com-

mon pollutants include salts, phosphates, hydrogen sulfide, and nitrogen compounds such as ammonia, nitrate, and nitrite (Iber and Kasan, 2021). These pollutants can originate from shrimp metabolic waste, uneaten feed, and dead shrimp. If discharged without adequate treatment, shrimp pond wastewater can degrade environmental quality. In particular, wastewater with high nitrogen and phosphate concentrations can cause eutrophication, triggering excessive algal growth that reduces water quality and dissolved oxygen levels and threatens aquatic life (Akinnawo, 2023).

To reduce the risk of environmental degradation from shrimp pond wastewater discharge, an effective treatment system is needed before releasing the wastewater into the environment. One approach is biological treatment, which uses microorganisms to degrade organic contaminants (Sharma et al., 2023). Biological wastewater treatment can be carried out using either activated sludge or biofilm systems. Biological wastewater treatment can use activated sludge or

biofilm systems. Activated sludge relies on floc-forming microorganisms to reduce Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), and other pollutants. It can effectively remove nitrogen and organic matter from wastewater (Wang et al., 2018). However, this system requires a continuous oxygen supply, produces sludge that requires further treatment, and involves complex operational and technical requirements. It may also be susceptible to sudden increases in pollutant loads (shock loading) and requires careful monitoring to maintain stable operation (Li et al., 2023). In contrast, biofilms consist of communities of microorganisms that grow and attach to surfaces or substrates in aquatic environments (Saini et al., 2023). Compared with conventional suspended-growth systems, biofilm systems can produce less sludge, provide more stable treatment performance, and retain microorganisms for longer periods because they are attached to a supporting surface (Masatlis et al., 2025). Various media, including Biogrow, can provide a surface for biofilm development.

Biogrow is a plastic wastewater treatment medium consisting of thin strips that provide a surface for biofilm-forming microorganisms to attach and grow. Each strip is approximately 0.3 cm wide and 14 cm long, providing a relatively large surface area for microbial attachment. Anwar et al. (2023), reported that Biogrow media combined with Greenbio 1103 Bacterial Resin reduced TSS by 64%, BOD₅ by 90.81%, and COD by 89.06% in pig manure through aerobic treatment. Greenbio 1103 Bacterial Resin is a semi-solid bioactive product containing a bacterial consortium that degrades organic pollutants. The consortium generally consists of bacteria involved in organic matter degradation and the nitrogen cycle. These findings demonstrate the potential of Biogrow combined with Greenbio 1103 Bacterial Resin for reducing pollutant concentrations in wastewater.

Previous studies have demonstrated the effectiveness of hybrid biofilm-activated sludge configurations for treating wastewater with challenging nutrient profiles. Wang et al. (2026), developed a coupled electrocoagulation-integrated fixed-film activated sludge (EC-IFAS) system for feed-based synthetic aquaculture wastewater with a low C/N ratio. They achieved stable total nitrogen removal efficiencies of 75.1–81.2%, along with high COD and turbidity removal. This performance was attributed to enhanced biomass retention and substrate utilization through the coexistence of suspended sludge and attached biofilm. Similarly, Tuo et al. (2024), demonstrated that an integrated fixed-film activated sludge (IFAS) system outperformed a conventional activated sludge system in removing COD, ammonia nitrogen, and total nitrogen. However, these previous studies mainly used synthetic wastewater formulated to simulate domestic sewage or aquaculture effluent. Experimental validation of hybrid treatment systems using strip-type biofilm carriers such as Biogrow combined with bacterial resin remains limited, particularly for actual shrimp pond wastewater. The characteristics of actual shrimp pond wastewater may

differ substantially from those of municipal or synthetic aquaculture wastewater. Therefore, this study aims to compare activated sludge, Biogrow, and a combination of both media to evaluate their effectiveness in reducing pollutant concentrations in shrimp pond wastewater.

2. EXPERIMENTAL SECTION

2.1 Materials

The tools used in this study included a 15-liter water gallon to serve as a wastewater treatment tank, a UV-Vis spectrophotometer, an oven, a magnetic stirrer, an analytical balance, a vacuum pump, a desiccator, an aerator, a thermometer, pH strips, and glassware.

Materials used in this study included shrimp pond wastewater collected from the retention pond of the Y shrimp farm in Kendal, Greenbio 1103 bacterial resin, and Biogrow media. Filtration was carried out using E-D Scientific Specialties grade 161 filter paper (VWR brand grade 161) with a pore diameter of 1.1 μm . Reagents used for water quality analysis included reagents for Chemical Oxygen Demand (COD), ammonia, nitrate, and phosphate measurements.

2.2 Methods

2.2.1 Research Design

This study used a completely randomized factorial design with two factors: treatment media and treatment duration. The experiment included four treatment media: a control treatment (without treatment media or bacterial resin), activated sludge (AS) derived from bacterial resin, Biogrow (BG) media, and a combination of AS and BG. The treatment duration consisted of five observation intervals: 0, 2, 4, 6, and 8 days. Each treatment was conducted in triplicate, resulting in a total of 60 experimental units. Sampling was carried out every two days from day 0 to day 8. Each sample included temperature, pH, total suspended solids (TSS), chemical oxygen demand (COD), phosphate, ammonia, and nitrate concentrations.



Figure 1. Biogrow Media Used in Wastewater Treatment

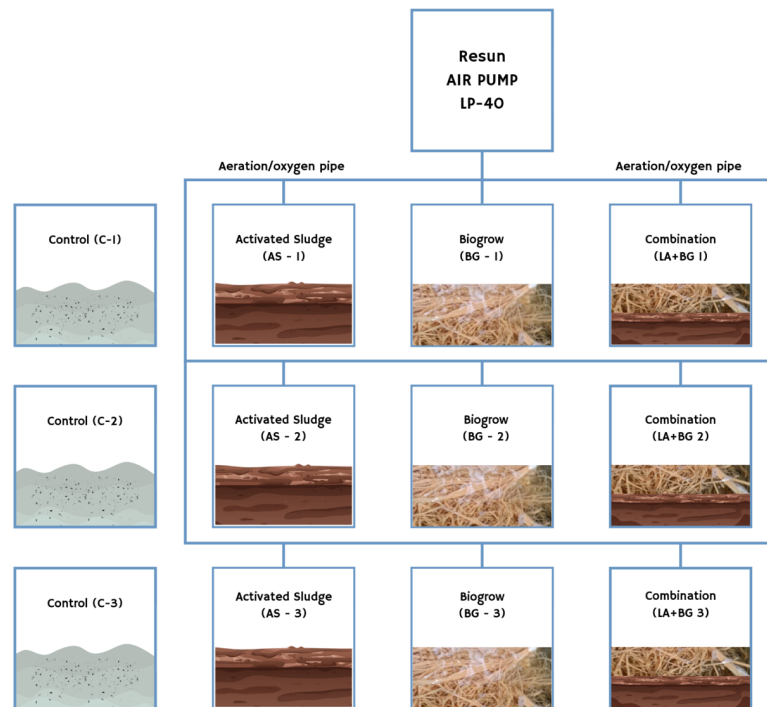


Figure 2. Research Design for Shrimp Farm Wastewater Treatment

The wastewater treatment system used in this study was a batch system, in which wastewater was treated in a single tank with no inflows or outflows. This study used a batch system conducted in a 15-liter water gallon container with a working volume of 5 L. The media ratio for the activated sludge and Biogrow treatments was 20% (v/v) of the total volume, while the combined system used 10% activated sludge and 10% Biogrow. To ensure aerobic conditions and fully mix the activated sludge and biofilm carriers, continuous aeration was provided using a Resun LP-40 air pump. Based on the pump's technical specifications (50 L/min capacity shared among 9 units), the estimated aeration rate was 5.5 L/min per tank. The Biogrow medium and the research design used are shown in Figures 1 and 2.

2.2.2 Bacterial Resin Cultivation and Acclimatization

This study used Greenbio 1103 resin bacteria. The resin was cultured in two cone-shaped containers, each containing 5 kg of resin and 6 L of tap water. The cone-shaped design ensured even oxygen distribution. The cultivation, or activation, process lasted two weeks, during which continuous aeration was maintained, and 600 mL of nutrients were added to each tank. The success of the activation phase was monitored through microscopic observation, which was indicated by the formation of flocs, along with the presence of live protozoa, particularly ciliates, actively moving within the floc matrix. The presence of motile ciliates was used as an additional indicator of a mature and metabolically

active microbial community, since protozoa generally colonize the system only after sufficient bacterial biomass has developed to sustain them as a food source. Once dense, well-formed flocs with actively moving ciliates were observed, the bacterial resin was considered ready and the process could advance to the next stage. In the first tank, the grown bacterial resin was attached to the Biogrow media, a process that took one week. Meanwhile, in the second tank, the grown bacterial resin was utilized for activated sludge treatment.

Once the resin was confirmed to have adhered to the Biogrow media and the activated sludge had grown effectively, the process moved on to acclimatizing the bacterial resin. This acclimatization process takes place over two days using a 1:1 mixture of tap water and wastewater, totaling 6 liters. Acclimatization ensures that the bacteria, whether attached to the Biogrow or present as activated sludge, can adapt to the wastewater before treatment.

2.2.3 Characterization of the Physical Characteristics of Shrimp Pond Wastewater

The physical parameters of wastewater analyzed in this study included Temperature and Total Suspended Solids (TSS). Temperature was measured at the time of sampling the shrimp pond wastewater and on the last day of the treatment period (D8). TSS testing was conducted on Day 0 and Day 8. The temperature was measured according to the method outlined in SNI 06-6989.23-2005, which details the procedure for testing temperature with a thermometer.

TSS testing followed the gravimetric method outlined in SNI 06-6989.3-2004.

2.2.4 Characterization of the Chemical Characteristics of Shrimp Pond Wastewater

This study analyzed pH, COD (Chemical Oxygen Demand), phosphate, ammonia, and nitrate. pH was measured at the time of sampling the shrimp pond wastewater and again on the last day of the treatment period (Day 8) using a pH strip. COD, phosphate, ammonia, and nitrate levels were measured on Days 0, 2, 4, 6, and 8. COD measurements were performed following the method outlined in SNI 6989.2:2009, which describes the Test Method for Chemical Oxygen Demand (COD) using closed-reflux spectrophotometry. Phosphate measurements were conducted according to the method specified in SNI 06-6989.31-2005 regarding the Test Method for Phosphate Content. This study measured ammonia using the Nessler method (Abdul-Rahaman, 2012). Nitrate measurements were conducted using the salicylic acid method, also known as the Cataldo method (Adonadaga, 2014).

2.2.5 Kinetic Modeling of Pollutant Removal

Kinetic modeling is important for understanding pollutant removal because it describes how TSS, ammonia, chemical oxygen demand (COD), and phosphate concentrations decrease during wastewater treatment. It also enables a quantitative comparison of the treatment performance of activated sludge (AS), Biogrow (BG), and combined (AS+BG) media. Kinetic information is particularly useful for estimating pollutant concentrations at different treatment times and determining the time required to achieve the targeted quality standard (Nemati et al., 2024). In this study, the removal kinetics of TSS, ammonia, COD, and phosphate for each treatment medium were evaluated using a first-order kinetic model, as presented in:

$$\ln C_t = \ln C_0 - kt \quad (1)$$

Where C_t is the concentration of ammonia, COD, or phosphate at time t (mg/L), C_0 is the initial concentration at $t = 0$ (mg/L), k is the first-order rate constant (day^{-1}), and t is the treatment time (days). For each parameter and treatment medium, the k value was obtained from the slope of the linear regression between $\ln C_t$ and t , and the coefficient of determination (R^2) was used to evaluate the model's goodness of fit.

The half-life ($t_{1/2}$), which represents the time required for the pollutant concentration to decrease to half of its initial value, was then calculated to facilitate comparison among the treatment media using:

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k} \quad (2)$$

A larger k value and a shorter $t_{1/2}$ indicate a faster pollutant removal process. These kinetic parameters there-

fore provide a quantitative basis for comparing the removal performance of AS, BG, and AS+BG.

2.2.6 Data Analysis

For data analysis, parametric statistics were employed since the data met the assumptions of normality and homogeneity. A two-way ANOVA was used to evaluate the interaction between waste-treatment time and treatment medium. Additionally, Tukey's post hoc test was utilized to identify significant differences among the treatments. Differences were considered significant at a p -value of less than 0.05.

3. RESULTS AND DISCUSSION

3.1 Normality Test, Homogeneity Test, and Interaction Effect

Before further statistical analysis, normality and homogeneity of variance were assessed for the physical and chemical parameters of the shrimp pond wastewater. The Shapiro-Wilk normality test indicated that all parameters were normally distributed, as evidenced by significance values greater than 0.05 ($p > 0.05$) (Table 1). Similarly, Levene's test for homogeneity of variance showed that most parameters met the assumption of homogeneity ($p > 0.05$) (Table 2). These results confirmed that the data satisfied the prerequisites for parametric statistical testing.

Table 1. Normality Test of Physical and Chemical Characteristics of Shrimp Pond Wastewater

Parameters	Statistic	df	Sig.
TSS	0.932	24	0.111
COD	0.961	60	0.051
Ammonia	0.972	60	0.181
Nitrate	0.972	60	0.192
Phosphate	0.972	60	0.181

Furthermore, a Two-Way ANOVA examined the interaction effect between treatment time and medium type for each parameter. As shown in Table 3, the interaction effect was highly significant ($p < 0.001$) for all parameters, indicating that the combined influence of the treatment duration and medium type significantly affected the reduction of TSS, COD, ammonia, nitrate, and phosphate concentrations in the shrimp pond wastewater.

3.2 Physical Characteristics of Shrimp Pond Wastewater

The evaluation of shrimp pond wastewater quality during treatment revealed physical and chemical characteristics before treatment (D0) and after 8 days of treatment (D8). Initially, temperature, pH, and nitrate concentration met the established quality standards (Table 4). However, total suspended solids (TSS), chemical oxygen demand (COD), ammonia, and phosphate exceeded the standards. After

Table 2. Test of Homogeneity of Variances (Levene's Test)

Parameters		Levene Statistic	df1	df2	Sig.
TSS (mg/L)	Based on Mean	1.022	7	16	0.453
	Based on Median	1.022	7	16	0.453
	Based on Median and with adjusted df	1.022	7	7.575	0.485
	Based on Trimmed Mean	1.022	7	16	0.453
COD (mg/L)	Based on Mean	1.734	19	40	0.071
	Based on Median	0.694	19	40	0.803
	Based on Median and with adjusted df	0.694	19	19.396	0.784
	Based on Trimmed Mean	1.652	19	40	0.090
Ammonia (mg/L)	Based on Mean	1.400	19	40	0.182
	Based on Median	0.869	19	40	0.619
	Based on Median and with adjusted df	0.869	19	17.839	0.619
	Based on Trimmed Mean	1.365	19	40	0.200
Nitrate (mg/L)	Based on Mean	1.579	19	40	0.111
	Based on Median	0.414	19	40	0.979
	Based on Median and with adjusted df	0.414	19	17.554	0.967
	Based on Trimmed Mean	1.465	19	40	0.152
Phosphate (mg/L)	Based on Mean	2.129	19	40	0.022
	Based on Median	0.908	19	40	0.577
	Based on Median and with adjusted df	0.908	19	13.818	0.586
	Based on Trimmed Mean	2.034	19	40	0.029

Table 3. Two-Way ANOVA Interaction Effects of Treatment Time and Medium Type on Water Quality Parameters

Parameters	Treatment Time×Medium		
	F	Sig.	Information
TSS (mg/L)	1401.408	0.000	Highly Significant
COD (mg/L)	73.710	0.000	Highly Significant
Ammonia (mg/L)	1282.327	0.000	Highly Significant
Nitrate (mg/L)	447.124	0.000	Highly Significant
Phosphate (mg/L)	907.416	0.000	Highly Significant

8 days of treatment, results showed that Biogrow media effectively reduced COD, ammonia, and phosphate levels. Additionally, nitrate concentration in the wastewater increased as ammonia concentration decreased during the treatment period.

The temperature of shrimp pond wastewater is strongly dependent on the intensity of sun exposure and the elevation of the pond site. The actual temperature of the shrimp pond wastewater measured during the day was recorded at 31 °C. This temperature is still within the normal range; according

to [Atikah et al. \(2023\)](#), the ideal temperature range for water in shrimp ponds is 25–31 °C. After 8 days of treatment, the temperature decreased to 28 °C across all treatments. Although the 8th-day measurement was also taken during the day, this temperature decrease was due to the treatment being conducted on a laboratory scale. This condition meant the wastewater was not directly exposed to sunlight, so its temperature was adjusted to room temperature.

Total Suspended Solids (TSS) are solid particles consisting of organic or inorganic materials suspended in water ([Adhar et al., 2022](#)). Measuring this parameter determines the amount of undissolved solids in wastewater ([Munandar et al., 2023](#)). If the concentration is too high, it can make the water turbid and reduce dissolved oxygen levels. In shrimp pond wastewater, high TSS levels are caused by the accumulation of uneaten feed and particles of shrimp feces ([Muqsith, 2014](#)). In this study, both the initial (D0) and final (D8) TSS concentrations exceeded the quality standards. The initial TSS concentration (D0) in this study was 980 mg/L, which was still lower than the value reported by [Sanjaya et al. \(2025\)](#), which reached 1723.33 mg/L before wastewater treatment. After 8 days of treatment, all treatments showed a decreasing trend in TSS. The decrease observed in the control treatment was due to sedimentation, or natural settling, driven by gravity. Based on the TSS data in Table 4, applying Biogrow (in both the BG and combination treatments) significantly reduced TSS compared

with the control and AS treatments.

This significant decrease indicates higher bacterial activity in the Biogrow media. It is assumed that the bacteria involved in this treatment process degrade organic pollutants and secrete Extracellular Polymeric Substances (EPS). According to [Melo et al. \(2022\)](#), EPS are biopolymeric compounds that act as biological adhesives, facilitating the aggregation of bacterial cells with each other and with other particulate matter, known as biofloculants. Biofloculants contain functional groups, such as hydroxyl, amino, and carboxyl groups, which act as adhesives that bind organic particles in wastewater ([Ugbenyen et al., 2015](#)). Although the shrimp pond wastewater was biologically treated, the TSS concentration on the final day still exceeded the quality standard, possibly due to biofilm release from the media surface, which was subsequently detected as suspended solids during sampling ([Arabgol et al., 2022](#)).

pH, or the potential of hydrogen, is a chemical parameter used to assess the acidity, neutrality, and alkalinity of a liquid or solution based on the concentration of hydronium ions ([Soares et al., 2019](#)). A low or high pH in wastewater can indicate the presence of contaminants. Additionally, knowing pH helps adjust it in biological wastewater treatment ([Syukor et al., 2021](#)). In this study, the initial pH of the shrimp pond wastewater was 8. After 8 days of treatment, the wastewater pH remained unchanged, likely due to the water's natural buffering capacity. Carbonate and bicarbonate ions provide this buffering and help maintain pH balance. During nitrification, hydrogen ions are released, which can acidify the water. However, residual alkalinity helps neutralize the acids produced during nitrification ([Biesterfeld et al., 2003](#); [Qi and Vielma, 2026](#)).

3.3 Chemical Characteristics of Shrimp Pond Wastewater

The two-way ANOVA revealed a significant interaction between treatment time and media type in changes in ammonia, COD, phosphate, and nitrate levels (Figures 3–6), where error bars represent the standard deviation (SD) of triplicate measurements ($n = 3$) and different lowercase letters indicate significant differences according to Tukey's HSD test at $p = 0.05$. At the start of the observation (D0), all treatments exhibited similar characteristics with no significant differences ($p > 0.05$), and their concentration levels were above the established quality standards. High concentrations of ammonia, COD, and phosphate resulted from the high organic load caused by the accumulation of feed residues and shrimp metabolic excretion products (feces) in the water ([Iber and Kasan, 2021](#)).

The initial characterization results aligned with several previous studies. The ammonia concentration recorded in this study was 15.18 mg/L, similar to [Mubarok and Zainuri \(2024\)](#), which reported ammonia levels in shrimp ponds reaching 14.28 mg/L. Similarly, the COD concentration of 1402.9 mg/L is comparable to results from [Sanjaya](#)

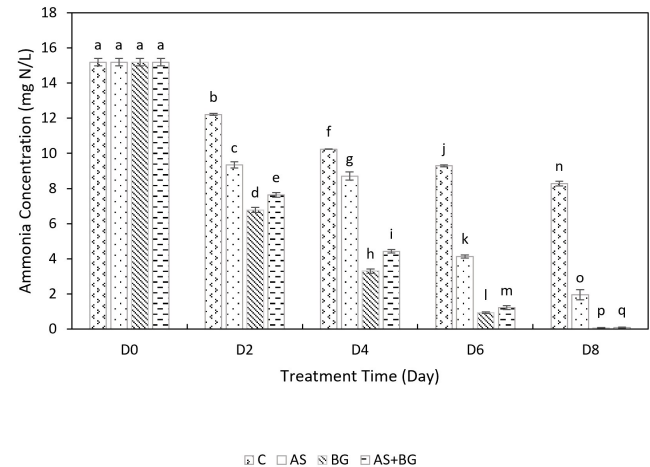


Figure 3. Concentration of Ammonia After 8 Days of Treatment

[et al. \(2025\)](#), which reported a COD of 1084.33 mg/L. The phosphate concentration of 1.27 mg/L is slightly above the 0.5–1 mg/L range reported by [Telaumbanua et al. \(2024\)](#). Discharging shrimp pond wastewater with ammonia, COD, and phosphate concentrations that exceed quality standards without prior treatment can cause environmental degradation. This may decrease dissolved oxygen levels and cause eutrophication, ultimately leading to physiological stress and inhibiting the growth of aquatic organisms ([Bai et al., 2023](#); [Sanjaya et al., 2025](#)).

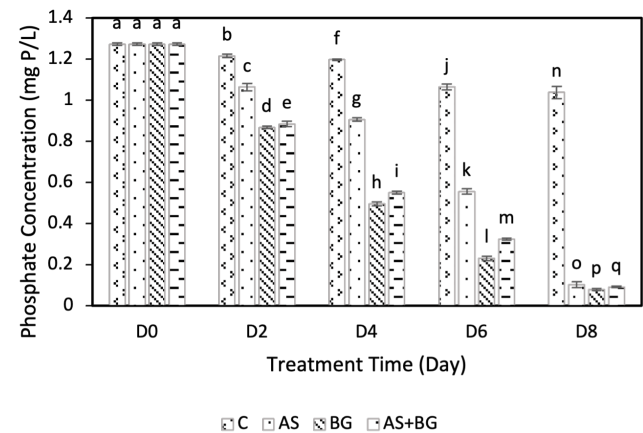


Figure 4. Concentration of Phosphate After 8 Days of Treatment

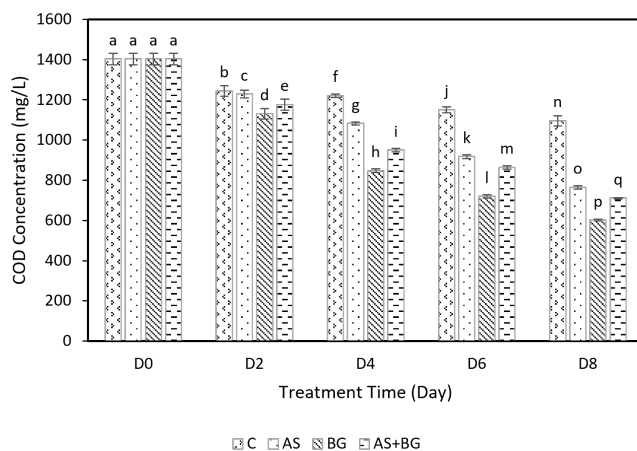
After 8 days of treatment, the concentrations of ammonia, chemical oxygen demand (COD), and phosphate decreased significantly ($p < 0.05$) across all experimental treatments compared to the control group (Figures 3–5). A statistically significant interaction was observed between the treatment duration and the type of treatment medi

Table 4. Physical and Chemical Characteristics of Wastewater from Shrimp Pond

Parameters	Unit	Concentration (Day 0)	Concentration (Day 8)				QS
			C	AS	BG	AS+BG	
Temperature	°C	31°C	28°C	28°C	28°C	28	25 – 31
pH	–	8	8	8	8	8	6 – 9*
TSS	mg/L	980 ± 3 ^a	730 ±3.6 ^b	740 ±4 ^b	560 ±6.25 ^c	600 ±4.35 ^c	≤ 200*
COD	mg/L	1402.9 ± 28.4 ^a	1096.2 ±25.6 ^b	765.1 ±8.4 ^c	601.7 ±5.1 ^e	710.6 ±3.3 ^d	100 – 250#
Ammonia	mg/L	15.18 ± 0.2 ^a	8.3 ±0.13 ^b	1.95 ±0.3 ^c	0.079 ±0.02 ^e	0.09 ±0.03 ^d	< 0.1*
Nitrate	mg/L	1.05 ± 0.06 ^e	1.89 ±0.06 ^d	2.26 ±0.1 ^c	6.88 ±0.04 ^a	4.92 ±0.09 ^b	< 75*
Phosphate	mg/L	1.27 ± 0.005 ^a	1.03 ±0.03 ^b	0.102 ±0.01 ^c	0.079 ±0.004 ^e	0.091 ±0.003 ^d	< 0.1*

Notes: C = control; AS = Activated Sludge; BG = Biogrow; AS+BG = Activated Sludge+Biogrow. QS = Quality Standards. Different lowercase letters indicate significant differences according to Tukey's HSD test at $p = 0.05$; the same letters indicate no significant difference. *Decree of the Minister of Marine Affairs and Fisheries No. 28 of 2004 concerning General Guidelines for Shrimp Pond Aquaculture. #Central Java Regional Regulation No. 5 of 2012 concerning Effluent Quality Standards for Businesses and/or Activities for Which Effluent Quality Standards Have Not Yet Been Established.

um ($p < 0.05$). This synergism is likely driven by the extended contact time between the immobilized bacterial consortium—whether hosted on activated sludge, Biogrow, or the combined AS+BG matrix—and the wastewater, which facilitates enhanced degradation of target pollutants. Mechanistically, microorganisms secrete specialized oxidative enzymes that break down complex organic contaminants into simpler, non-toxic compounds over time, thereby mitigating overall toxicity (Cao et al., 2022; Unuofin et al., 2019). The catalytic efficiency of these microbial enzymes is reflected in the accelerated rate of substrate-to-product conversion per unit of time as contact time progresses.

**Figure 5.** Concentration of COD After 8 Days of Treatment

The COD concentration in this study remains above the established quality standards. This is likely because a single biological treatment method has limited capacity to reduce the organic load in shrimp pond wastewater, especially when not combined with other methods. According to Derakhshan et al. (2023), biological wastewater treatment alone has limitations in producing water treated enough for direct discharge. Persistently high COD may also result from compounds that bacteria find difficult to degrade (Zhang et al., 2020). However, this study did not experimentally evaluate strategies to improve COD removal beyond the biological treatment configurations tested, which limits our ability to confirm the cause of the residual COD or identify effective mitigation approaches. Future studies should add a treatment step, such as physical, chemical, or physicochemical pretreatment (e.g., coagulation-flocculation or electrocoagulation), to remove particulate organic matter before biological treatment (Wang et al., 2026). Alternatively, a post-treatment polishing step could verify whether COD removal meets discharge standards. Additionally, future research could focus on optimizing operational parameters, such as extending hydraulic retention time or increasing the biofilm carrier ratio, to determine whether limitations stem from insufficient contact time or biomass capacity rather than solely from organic compounds that are difficult to degrade.

In contrast to the decreasing levels of ammonia, COD, and phosphate, nitrate concentrations significantly increased with the duration of wastewater treatment (Figure 6). Nitrate concentrations on day 0 (before treatment) and day 8 (after treatment in each medium) remained below the quality standards. The trend of increasing nitrate con-

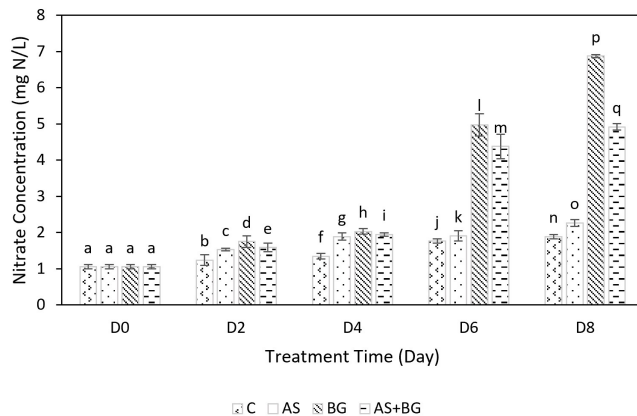


Figure 6. Concentrations of Nitrate After 8 Days of Treatment

centrations alongside decreasing ammonia concentrations during treatment reflects nitrification. Aeration, or oxygen supply, triggers ammonia oxidation through nitrification. In this process, ammonia is oxidized into nitrite, which is then converted into nitrate (Lu et al., 2021). The continuous accumulation of nitrate throughout the treatment period, without a subsequent decline, suggests that denitrification, the process by which nitrate is reduced to nitrogen gas, did not occur to a significant extent under the experimental conditions applied in this study. This is presumably attributed to the conditions in tanks with medium treatment. Denitrification is predominantly carried out by facultative anaerobic bacteria that require an anoxic environment to utilize nitrate as an electron acceptor (Pold et al., 2025). Under continuously aerated conditions, dissolved oxygen likely remained high enough to inhibit denitrifying bacteria, resulting in nitrate accumulation as the final product of nitrification rather than its further reduction (Urbini et al., 2015). High concentrations of nitrogen and phosphorus in wastewater can trigger eutrophication (Musifa et al., 2025). Eutrophication can cause algal blooms or accelerate phytoplankton growth in water bodies. Algal blooms can degrade water quality and reduce dissolved oxygen levels, thereby disrupting the survival of other organisms in the water (Akinawo, 2023).

3.4 Performance of Biogrow Media in Pollutant Removal

Based on reductions in ammonia, chemical oxygen demand (COD), and phosphate concentrations, the Biogrow (BG) media showed the greatest reduction. This was followed by the mixed media (AS+BG), while the activated sludge media showed the least reduction. This difference is attributed to the superior characteristics of the biofilm formed on the Biogrow (BG) media compared to the suspended system used in activated sludge. According to Chinnaraj et al. (2014), the biofilm's layered structure physically pro-

tections the microorganisms inside, providing greater resistance to toxic substances and fluctuations in wastewater load (shock loads). In contrast, bacteria in activated sludge are more vulnerable to pollutants because their cells are freely dispersed in the water and directly exposed to contaminants. This study evaluated pollutant removal using shrimp pond wastewater with relatively stable initial characteristics. Consequently, it did not experimentally compare the performance of Biogrow, activated sludge, and the combined media under varying wastewater conditions or sudden increases in pollutant loads (shock loads). The study inferred the Biogrow biofilm's greater resistance to shock loads from its structural characteristics and supporting literature, rather than directly verifying it. Future research should assess the performance and stability of these treatment media under fluctuating pollutant concentrations and shock-loading conditions. Moreover, the high pollutant removal efficiency of the Biogrow (BG) media can also be attributed to its larger specific surface area. Each Biogrow strip used in this study measured approximately 0.3 cm in width and 14 cm in length, resulting in an estimated surface area of about 8.4 cm² per strip (both sides). Due to the many strips in a Biogrow medium, the total available attachment area for biofilm formation was significantly greater than the surface area provided by the flocs in the suspended activated sludge system. According to Boavida-Dias et al. (2022), adding media to biological treatment systems can significantly increase the total available area for bacteria to grow, attach, and form biofilms. A larger surface area improves wastewater treatment efficiency because more bacteria can treat pollutants in the wastewater.

3.5 Kinetic Analysis of Pollutant Removal

To quantitatively strengthen comparisons of treatment performance beyond pollutant removal, first-order kinetic modeling was applied to the TSS, COD, ammonia, and phosphate removal data for each treatment medium (Table 5). The regression models showed a strong fit to the first-order kinetic equation, with R^2 values ranging from 0.78 to 1.00 for most parameters and treatments, confirming that pollutant removal in this study largely followed first-order kinetics.

The Biogrow (BG) medium consistently showed the highest rate constant (k) across all four parameters, indicating the fastest pollutant removal among the treatments. Its short half-life ($t_{1/2}$), particularly for ammonia (1.10 days compared with 9.34 days in the control), further demonstrates its superior removal efficiency. This suggests that Biogrow's larger specific surface area and layered biofilm structure enhanced pollutant removal and accelerated degradation. The higher k value for ammonia (0.6294) than COD (0.1072) indicates faster ammonia removal and may reflect favorable development and activity of nitrifying microorganisms. However, microbial composition and specific nitrification activity were not directly assessed. The AS+BG treatment generally showed intermediate k values, with im-

Table 5. First-Order Kinetic Model Parameters for Pollutant Removal

Parameters	Treatments	First-Order Rate	K (day ⁻¹)	T _{1/2} (days)	R ²
TSS (mg/L)	Control	Y=6.88755-0.03681x	0.03681	18.82641	1
	AS	Y=6.88755-0.03511x	0.03511	19.73797	1
	BG	Y=6.88755-0.06995x	0.06995	9.907076	1
	AS+BG	Y=6.88755-0.06133x	0.06133	11.29953	1
COD (mg/L)	Control	Y=7.21971-0.02857x	0.02857	24.25621	0.94
	AS	Y=7.26244-0.07523x	0.07523	9.211751	0.99
	BG	Y=7.22844-0.10724x	0.10724	6.462141	0.99
	AS+BG	Y=7.23376-0.08343x	0.08343	8.306365	0.99
Ammonia (mg/L)	Control	Y=2.67552-0.07419x	0.07419	9.340880	0.97
	AS	Y=2.82540-0.24608x	0.24608	2.816157	0.94
	BG	Y=3.15365-0.62944x	0.62944	1.100979	0.92
	AS+BG	Y=3.21669-0.60079x	0.60079	1.153481	0.90
Phosphate (mg/L)	Control	Y=0.25092-0.02707x	0.02707	25.6003	0.93
	AS	Y=0.59737-0.28066x	0.28066	2.46918	0.78
	BG	Y=0.45591-0.34378x	0.34378	2.015824	0.96
	AS+BG	Y=0.45474-0.31373x	0.31373	2.208906	0.93

proved removal kinetics compared with AS alone but lower effectiveness than BG alone. The high R^2 values (> 0.90) across most parameters indicate that pollutant removal was well described by a first-order kinetic model, consistent with (Li et al., 2020), who reported $R^2 > 0.94$ for NH_4^+ -N removal across tested carriers and found that the first-order model adequately simulated NH_4^+ -N removal in SBBRs. Under first-order conditions, removal is concentration-dependent, with the removal rate decreasing as the remaining substrate concentration declines.

4. CONCLUSIONS

The analyzed wastewater from the shrimp farm showed total suspended solids (TSS), chemical oxygen demand (COD), ammonia, and phosphate concentrations that exceeded quality standards; in contrast, temperature, pH, and nitrate levels remained within acceptable limits. After 8 days of treatment, pollutant concentrations decreased significantly, with the degree of reduction varying by media type. Biogrow media achieved the best results, with its layered structure and high surface area promoting bacterial growth and biofilm formation. This medium is also more resilient to toxic substances and fluctuations in wastewater load. While biological treatment can effectively reduce pollutants in shrimp farm wastewater, the COD concentration at the end of the observation period was still above the quality standard. This indicates that biological treatment alone has limitations in breaking down complex compounds. Therefore, it is recommended to combine biological treatment with advanced methods, such as anaerobic biological, chemical, or physical treatments. However, this study was limited by the lack of continuous DO monitoring and detailed operational parameters, such as aeration rate and treatment container dimensions. Future studies should therefore incorporate

these measurements and evaluate additional treatment or operational strategies to improve COD removal further.

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REFERENCES

- Abdul-Rahaman, I. (2012). Managing and Maximising the Use of Sewage in Tamale. *International Knowledge Sharing Platform*, **2**(8); 41–51
- Adhar, S., Lukman, M. Khalil, E. Ayuzar, Erlangga, R. Rusydi, Mainisa, and Muliani (2022). Influence of Rainfall and Spatial Temporal Distribution Analysis of Total Suspended Solid in Laut Tawar Lake. *IOP Conference Series: Earth and Environmental Science*, **1062**(1); 012022
- Adonadaga, M.-G. (2014). Nutrient Removal Efficiency of Activated Sludge Plants Treating Industrial and Municipal Wastewater in Ghana. *Journal of Environment Pollution and Human Health*, **2**(3); 58–62
- Akinawo, S. O. (2023). Eutrophication: Causes, Consequences, Physical, Chemical and Biological Techniques for Mitigation Strategies. *Environmental Challenges*, **12**; 100733

- Aktur, S. and N. Yeumine (2020). Vannamei Shrimp Cultivation Including Distribution, Feed Quality and Feeding Method. *Journal Siplieria Sciences*, **1**(2); 1–5
- Anwar, C., A. N. Confera, A. S. Noerwahju, W. Handayani, and Y. A. Handoko (2023). The Evaluation of Physical, Biological, and Chemical Treatments on Piggery Wastewater: A study on pig farming in Sumogawe village, Semarang Regency, Central Java. *IOP Conference Series: Earth and Environmental Science*, **1275**(1); 012032
- Arabgol, R., P. A. Vanrolleghem, and R. Delatolla (2022). Influence of MBBR Carrier Geometrical Properties and Biofilm Thickness Restraint on Biofilm Properties, Effluent Particle Size Distribution, Settling Velocity Distribution, and Settling Behaviour. *Journal of Environmental Sciences*, **122**; 138–149
- Atikah, S. K., R. Kurniawan, and M. S. Hasibuan (2023). Litopenaeus Vannamei Shrimp Pond Water Temperature and pH Monitoring System Using IoT-Based Sugeno Fuzzy Method. *Jurnal Info Sains: Informatika dan Sains*, **13**(02); 393–398
- Bai, D., X. Li, Z. Liu, L. Wan, C. Song, Y. Zhou, and X. Cao (2023). Nitrogen and Phosphorus Turnover and Coupling in Ponds with Different Aquaculture Species. *Aquaculture*, **563**; 738997
- Biesterfeld, S., G. Farmer, P. Russell, and L. Figueroa (2003). Effect of Alkalinity Type and Concentration on Nitrifying Biofilm Activity. *Water Environment Research*, **75**(3); 196–204
- Boavida-Dias, R., J. R. Silva, A. D. Santos, R. C. Martins, L. M. Castro, and R. M. Quinta-Ferreira (2022). A Comparison of Biosolids Production and System Efficiency between Activated Sludge, Moving Bed Biofilm Reactor, and Sequencing Batch Moving Bed Biofilm Reactor in the Dairy Wastewater Treatment. *Sustainability*, **14**(5); 2702
- Cao, Z., W. Yan, M. Ding, and Y. Yuan (2022). Construction of Microbial Consortia for Microbial Degradation of Complex Compounds. *Frontiers in Bioengineering and Biotechnology*, **10**; 1051233
- Chinnaraj, S., M. Jothilingam, and S. Jeganathan (2014). An Evaluation of Biofilm Membrane Bioreactor (Bf-MBR). *IOSR Journal of Mechanical and Civil Engineering*, **11**(2); 05–11
- Derakhshan, A., R. Rezaei Kalantari, M. Farzadkia, A. Tiyuri, and A. Esrafil (2023). The Effect of Biological Treatment Methods on the Concentration of Carbonaceous Pollutants in the Slaughterhouse Wastewater: A Systematic Review. *Case Studies in Chemical and Environmental Engineering*, **8**; 100451
- Iber, B. T. and N. A. Kasan (2021). Recent Advances in Shrimp Aquaculture Wastewater Management. *Heliyon*, **7**(11); e08283
- Li, D., Z. Wang, Y. Yang, H. Liu, S. Fang, and S. Liu (2023). Research Status and Development Trend of Wastewater Treatment Technology and Its Low Carbonization. *Applied Sciences*, **13**(3); 1400
- Li, J., W. Zhu, H. Dong, Z. Yang, P. Zhang, and Z. Qiang (2020). Impact of Carrier on Ammonia and Organics Removal from Zero-Discharge Marine Recirculating Aquaculture System with Sequencing Batch Biofilm Reactor (SBBR). *Environmental Science and Pollution Research*, **27**(28); 34614–34623
- Lu, J., Y. Hong, Y. Wei, J.-D. Gu, J. Wu, Y. Wang, F. Ye, and J.-G. Lin (2021). Nitrification Mainly Driven by Ammonia-Oxidizing Bacteria and Nitrite-Oxidizing Bacteria in an Anammox-Inoculated Wastewater Treatment System. *AMB Express*, **11**(1); 158
- Masatlis, I., A. Chatzis, and A. Zouboulis (2025). Biofilm Control in Wastewater Treatment: A Review Regarding the Application of Quorum Sensing and Quenching Processes and Future Perspectives. *Water*, **18**(1); 77
- Melo, A., C. Quintelas, E. C. Ferreira, and D. P. Mesquita (2022). The Role of Extracellular Polymeric Substances in Micropollutant Removal. *Frontiers in Chemical Engineering*, **4**; 778469
- Mubarak, A. K. U. and M. Zainuri (2024). Efektifitas Rumput Laut (*Gracilaria* sp.) Terhadap Penurunan Nilai Kandungan Amonia dari Air Limbah Tambak Udang di Socah Madura. *Juvenil: Jurnal Ilmiah Kelautan dan Perikanan*, **5**(1); 96–103. (in indonesia)
- Munandar, A., K. Nabila, and R. N. Azizah (2023). Chemical Oxygen Demand (COD) and Total Suspended Solid (TSS) Removal from Rubber Wastewater Factory Using Electrocoagulation Technique. *Indonesian Journal of Environmental Management and Sustainability*, **7**(1); 27–31
- Muqith, A. (2014). Dampak Kegiatan Tambak Udang Intensif Terhadap Kualitas Fisik-Kimia Perairan Banyuputih Kabupaten Situbondo. *Samakia: Jurnal Ilmu Perikanan*, **5**(1). (in indonesia)
- Musifa, E., N. R. Palapa, and B. R. Ahadito (2025). Microalgae-Based Wastewater Treatment as Green Solution for Sustainable Degradation and Its Challenges: A Review. *Indonesian Journal of Environmental Management and Sustainability*, **9**(4); 171–182
- Nemati, B., M. M. Baneshi, H. Akbari, R. Dehghani, and G. Mostafaii (2024). Phytoremediation of Pollutants in Oil-Contaminated Soils by *Alhagi camelorum*: Evaluation and Modeling. *Scientific Reports*, **14**(1); 5502
- Pold, G., A. Saghaï, C. M. Jones, and S. Hallin (2025). Denitrification Is a Community Trait with Partial Pathways Dominating across Microbial Genomes and Biomes. *Nature Communications*, **16**(1); 9495
- Qi, W. and J. Vielma (2026). Effects of Alkalinity-Controlling Chemicals on Biofilter Performance and Sludge Properties in Freshwater Recirculating Aquaculture Systems. *Aquacultural Engineering*, **114**; 102726
- Saini, S., S. Tewari, J. Dwivedi, and V. Sharma (2023). Biofilm-Mediated Wastewater Treatment: A Comprehensive Review. *Materials Advances*, **4**(6); 1415–1443
- Sanjaya, E. H., A. A. Asha, Suharti, Muntholib, and H. Chen (2025). Effectiveness of Aerobic Microbes in the Treat-

- ment of Shrimp Pond Wastewater. *Malaysian Journal of Chemistry*, **27**(1); 211–219
- Sharma, M., S. Agarwal, R. Agarwal Malik, G. Kumar, D. B. Pal, M. Mandal, A. Sarkar, F. Bantun, S. Haque, P. Singh, N. Srivastava, and V. K. Gupta (2023). Recent Advances in Microbial Engineering Approaches for Wastewater Treatment: A Review. *Bioengineered*, **14**(1); 2184518
- Soares, H. S. A., F. Rocha Pinto, D. B. d. Alencar, and M. H. R. Nascimento (2019). pH Analysis of a Wastewater Treatment Plant (WWTP) in a Paper Recycling Industry. *International Journal for Innovation Education and Research*, **7**(11); 1062–1069
- Syukor, A. R. A., S. Sulaiman, J. P. Chandrakant, P. Mishra, M. Nasrullah, L. Singh, and A. W. Zularism (2021). Energy Generation from Fish-Processing Waste Using Microbial Fuel Cells. In *Delivering Low-Carbon Biofuels with Bioproduct Recovery*. Elsevier, pages 101–121
- Telaumbanua, B. H., N. P. L. Pangemanan, R. A. Tumbol, S. L. Undap, S. S. Tumembouw, and I. R. N. Salindeho (2024). Fluktuasi Parameter Kualitas Air di Tambak Super Intensif Udang Vaname: Studi Kasus Pertambakan PT. Pillar Persada Parigi, Desa Bajo, Minahasa Selatan. *Budidaya Perairan*, **12**(2); 72–79. (in indonesia)
- Tuo, Z., L. Bai, B. Zhang, S. Jing, C. Li, and S. Tang (2024). Characteristics of Nitrogen Removal from an Integrated Fixed-Film Activated Sludge (IFAS) System and the Relationship Between Activated Sludge and Biofilm Interactions. *Water*, **16**(21); 3040
- Ugbenyen, A., N. Vine, J. Simonis, A. Basson, and A. Okoh (2015). Characterization of a Biofloculant Produced from the Consortium of Three Marine Bacteria of the Genera Cobetia and Bacillus and Its Application for Wastewater Treatment. *Journal of Water, Sanitation and Hygiene for Development*, **5**(1); 81–88
- Unuofin, J. O., A. I. Okoh, and U. U. Nwodo (2019). Aptitude of Oxidative Enzymes for Treatment of Wastewater Pollutants: A Laccase Perspective. *Molecules*, **24**(11); 2064
- Urbini, G., R. Gavasci, and P. Viotti (2015). Oxygen Control and Improved Denitrification Efficiency by Means of a Post-Anoxic Reactor. *Sustainability*, **7**(2); 1201–1212
- Wang, J., Y. Qian, and J. Lv (2026). Coupled Electrocoagulation-Integrated Fixed-Film Activated Sludge Process for Treatment of Low C/N Ratio Aquaculture Wastewater. *Environmental Technology & Innovation*, **42**; 104913
- Zhang, B., D. Ning, Y. Yang, J. D. Van Nostrand, J. Zhou, and X. Wen (2020). Biodegradability of Wastewater Determines Microbial Assembly Mechanisms in Full-Scale Wastewater Treatment Plants. *Water Research*, **169**; 115276