

Performance of an anaerobic co-digestion system treating commercial remazol blue rsp dye and real honey wastewater: decolorization, stability, and detoxification

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Abstract

Background: This study aimed to evaluate the performance of an anaerobic co-digestion (AcoD) system in dye detoxification, decolorization, and chemical oxygen demand (COD) removal.

Methods: Herein, synthetic textile (Remazol blue RSP) wastewater (50, 100, and 150 mg/L) and real honey wastewater were anaerobically co-digested using an anaerobic up-flow reactor at a hydraulic retention time (HRT) of 24 h and a C:N ratio of 5:1. Total alkalinity (TA) and volatile fatty acid (VFA) were measured using spectrophotometric method, while color removal was monitored at λ max 595 nm. COD removal was measured using the closed reflux titrimetric method. The phytotoxicity test was conducted based on germination percentage, relative toxicity, and *Phaseolus vulgaris* plumule length.

Results: COD removal at 50, 100, and 150 mg/L dye concentrations was relatively similar, as $76.69 \pm 1.33\%$, $75.91 \pm 2.1\%$, and $75.77 \pm 2.83\%$, respectively. The decolorization rate decreased with the increase in dye concentration from $97.37 \pm 2.18\%$ to $92.22 \pm 0.51\%$ and $90.12 \pm 0.49\%$. VFA/(TA) ratio markedly increased with increasing dye concentration, from 0.05 to 0.32 and 0.35, indicating system instability. Additionally, the phytotoxicity test revealed that the AcoD system improved the effluent quality and reduced dye wastewater toxicity.

Conclusion: The findings suggest that AcoD of textile and honey wastewater can effectively remove pollutants (COD), detoxify, and decolorize Remazol Blue RSP-containing synthetic wastewater. Upon increasing dye concentration, a decrease in decolorization rate and system instability were observed, reflecting the effect of dye toxicity on the anaerobic system.

Keywords: Coloring agents, Digestion, Environmental pollutants, Textile, Wastewater

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Introduction

Dyes used in textile production contribute considerably to the large quantity and toxicity of textile wastewater. Due to its carcinogenic, mutagenic, and teratogenic characteristics, textile wastewater poses serious environmental and human health risks if improperly disposed of. Metals, chlorides, and intermediate byproducts of dye degradation exhibit toxic effects in aquatic ecosystems. Therefore, establishing an effective method for treating textile wastewater is crucial for environmental and ecological concerns (1–3).

Physical processes, such as coagulation-flocculation and filtration, have been extensively studied for treating textile

wastewater because of their simplicity and conventional nature. Furthermore, chemical processes coupled with enzymatic treatments have been investigated to enhance the decolorization and degradation of textile wastewater, resulting in high stability. Moreover, an integrated system that degrades the azo dye using activated carbon adsorption and bioelectrochemical processes has been reported (4,5). However, these methods present various limitations regarding full-scale implementation, including high-cost maintenance, generation of hazardous side products as secondary pollutants, and complex adsorbent regeneration.

Additionally, biological processes, specifically anaerobic



digestion (AD), have been studied and implemented as an alternative to physical methods. Previous studies have combined anaerobic sludge and zero-valent iron for Reactive Blue 13 decolorization (6). Additionally, anaerobic sequencing batch reactors have been reported to decolorize Orange II without co-substrates (7). Reportedly, Wijetunga et al effectively achieved a decolorization rate of up to 85% for acid dyes at different concentrations using an up-flow anaerobic sludge blanket (UASB) reactor (8). Holkar et al reported excellent decolorization of the AD system using *Enterobacter* sp. and glucose supplementation (9), and Malik et al achieved 97% decolorization and 77% chemical oxygen demand (COD) removal in synthetic textile wastewater using an AD system (10). These studies indicate the potential of AD as a viable method for treating textile wastewater.

However, the performance and stability of AD using a single substrate have been reported to be low owing to an insufficient C:N ratio, especially for toxic and recalcitrant pollutants such as dyes. Hence, the anaerobic co-digestion (AcoD) system is preferred to increase biomass conversion efficiency. Owing to its advantages of adjusting the C:N ratio, increasing biogas production, and improving efficiency, the AcoD system has gained considerable popularity (11). AcoD has been widely used to enhance the biogas production of anaerobic digesters. It utilizes two or more substrates with complementary characteristics to improve biogas production and promote system stabilization through the synergistic effects of microorganisms and nutrient balance. Substrates used in the AcoD system are usually solid waste, such as food waste, crop waste, brown water, and waste-activated sludge (12–17). However, pretreatment for solid waste is generally required and can incur extra costs. On the other hand, the AcoD system can utilize wastewater as a substrate, thereby potentially reducing pollutant toxicity and improving degradation efficiency. Thus, in this study, the possibility of employing wastewater as a substrate in the AcoD system was evaluated to minimize the negative effect of toxic (dye) wastewater on the stability of AD. Herein, the AcoD of synthetic dye and real honey wastewaters was performed, and the impact of dye concentrations on decolorization rate, COD removal, system stability, and effluent toxicity was investigated.

Materials and Methods

Synthetic textile wastewater

Herein, the synthetic textile wastewater was prepared by simulating the industrial dyeing process to imitate the actual composition of dyeing textile wastewater. Commercial Remazol Blue RSP was used to prepare the synthetic wastewater for all experiments (Figure S1). Remazol brilliant blue R (50 mg/L), Na₂CO₃ (1300 mg/L; Ansac, USA), and NaCl (150 mg/L; Merck, Germany) were dissolved in Aquadest, and the mixed solution was

then heated at 80°C for 30 min. Following this, 150 mg/L dye was mixed to prepare the stock solution. The stock was then diluted to obtain influent wastewater with 50 and 100 mg/L dye concentrations (10). Honey wastewater was obtained from a honey-processing industry and used without any pretreatment. Herein, the anaerobic granule used as inoculum was obtained from the anaerobic reactor used in our previous study (10) at 30% w/v.

Experimental setup and procedure

To assess the operational and performance characteristics of AcoD, two laboratory-scale cylinder UASB reactors with a total working volume of 4.8 L were prepared, with sampling ports provided at the top (Figure S2). The effects of synthetic textile wastewater with 50, 100, and 150 mg/L dye concentrations on the decolorization rate, COD removal, and AcoD stability were investigated (Table 1). Honey-processing wastewater was used as substrate at a 5:1 C:N ratio, which was the best configuration in our previous study (10). Additionally, the influent was fed into the reactor using a peristaltic pump (Masterflex C/L, Cole-Perkin, Barrington, USA) with an adjusted flow rate at a hydraulic retention time (HRT) of 24 h. The characteristics of raw synthetic wastewater are presented in Table S1.

Bacterial adsorption

Raw granules were used to investigate the adsorption mechanism of dye onto the granules. They were thoroughly cleaned to eliminate debris and dirt and filtered using a 0.5-mm diameter stainless steel sieve. For the inactivation of microorganisms, granules were subjected to a water bath at 100°C for 30 min to ensure no morphological alterations occurred. Following this, 5 and 10 g of inactive granules were inoculated into Erlenmeyer flasks and fed with synthetic wastewater with 50 mg/L dye concentration without honey-processing wastewater or external nitrogen to prevent any possible microbial activity. The flasks were placed in a rotary shaker operating at 150 rpm and room temperature. Finally, the granules were visualized using a digital microscope at 35× and 100× magnifications (Hirox KH 8700).

Phytotoxicity analysis

The treated wastewater was subjected to the phytotoxicity analysis (Table S2). The seeds of red bean (*Phaseolus vulgaris*; five beans, average weight = 2 ± 0.2 g) were used to evaluate the residual toxicity of AcoD-treated wastewater. Seeds were placed evenly in 250 mL plastic bowls containing sterile cotton and filter paper. Next, 5 mL of the sample was added to each bowl, and Aquadest was used as a control. Bowls were then closed to stimulate sprouting at 28°C ± 2°C (18). After 7 days, the quantity of germinated seeds and the length of the radicle and plumule were recorded. Each variable was tested in

Table 1. Operational condition and experimental setup

Dye concentration (mg/L)	Average COD influent	Average OLR (g/COD/L/day)	C:N ratio	HRT (h)
50	620.85±19.38	0.62±0.019	5:1	24
100	679.57±2.41	0.67±0.002	5:1	24
150	627.72±10.13	0.62±0.01	5:1	24

triplicate. Germination percentage and seedling length were measured to calculate the relative toxicity (%RT; Eq. 1) of treated wastewater (19) and effluent germination index (GI; Eq. 2) (20), as follows:

$$RT(\%) = \frac{C-Y}{C} \times 100 \quad (1)$$

where C and Y refer to the quantity of germinated seeds or seedling length in the control after 7 days of incubation and treating wastewater, respectively.

$$GI = 100 \times \frac{Gs}{Gc} \times \frac{Ls}{Lc} \quad (2)$$

where Gs and Gc represent the number of germinated seeds in the sample and control groups, respectively. Ls and Lc indicate the length of the roots (radicles) in the sample and control groups, respectively.

Analytical methods

Samples were collected daily in duplicates from the outlet port using plastic bottles. Total alkalinity (TA) was measured based on the CaCO_3 (mg/L) content using a Gallery plus automated chemistry analyzer (Thermo Fisher Scientific, Finland). Volatile fatty acid (VFA) was measured using a previously described spectrophotometric method (21). Color removal was calculated as the difference in absorbances of the effluent and influent at the maximum wavelength of 595 nm. The absorbance spectrum scan was measured using a UV-visible spectrophotometer (Agilent Cary 60 UV-Vis, Malaysia). To eliminate the interference of solids, all samples were filtered using Whatman grade 1 filter paper (Cytiva, England). COD was measured using a closed reflux titrimetric method per the Standard Method (22).

Results

Granular sludge morphology

The morphology of granular sludge used in this study was analyzed using a digital microscope. The crater structure of the granular sludge was visible (Figure 1). Grayish-white and black colors could be noticed on the outer layer and in the center of the granule.

Adsorption capacity of granular sludge

Figure 2 presents the adsorption capacity of the granular sludge used in this study. The granular sludge presented a relatively low adsorption capacity with a maximum decolorization of 21.80% and 17.97% at 5 g and 10 g granules, respectively. Decolorization performance by

granular adsorption did not increase significantly after 24 h, reaching 41.15% and 31.76% at 5 g and 10 g granules, respectively.

Granule morphology after the adsorption experiment was observed at 100× magnification (Figure 3). Notably, the dye was adsorbed into granular matrices, most deposited in the outer granular layer, whereas only a small portion reached the center.

Decolorization performance

Herein, the AcoD system presented a relatively high and stable decolorization performance of 96–98%. However, it was considerably reduced to 91–93% at 100 mg/L dye concentration and further decreased to 89.19% at 150 mg/L dye concentration (Figure 4). The absorbance spectrum scan supported the enhanced decolorization performance of Remazol blue RSP. The intense color of Remazol Blue RSP was represented by peaks at 595 nm (Figure S3).

The dye degradation performance of the AcoD system at different inlet concentrations is presented in Table 2. The AcoD system could remove the dye up to 648.86 mg/day, which was lower than a previously reported result (23), in which 883 mg/day of dye degradation was achieved using a hollow fiber membrane bioreactor.

COD removal efficiency

The COD removal efficiency of the AcoD system at higher dye concentrations was relatively similar; however, it decreased slightly at a dye concentration of 150 mg/L (Figure 5). At a dye concentration of 50 mg/L, COD removal fluctuated, ranging from 73.22% to 80.39%. Subsequently, albeit lower, stable COD removal was observed, ranging from 73.02% to 77.62% at an increased dye concentration of 100 mg/L. At a dye concentration of 150 mg/L, COD removal fluctuated after the fifth day, ranging from 72.01% to 78.69%.

The average COD removal at different dye concentrations was not significantly different ($P > 0.05$) (76.69%, 75.91%, and 75.77% at 50, 100, and 150 mg/L, respectively) (Figure 6).

All experiments in this study were performed at a fixed C:N ratio of 5:1 and an HRT of 24 h, which were reported as the optimum C:N ratio and HRT for the anaerobic co-digestion of Remazol blue RSP based on the best COD degradation, decolorization, and system stability (10). Additionally, the effect of increasing dye concentrations was investigated to analyze the optimum performance and system stability at high dye concentrations while

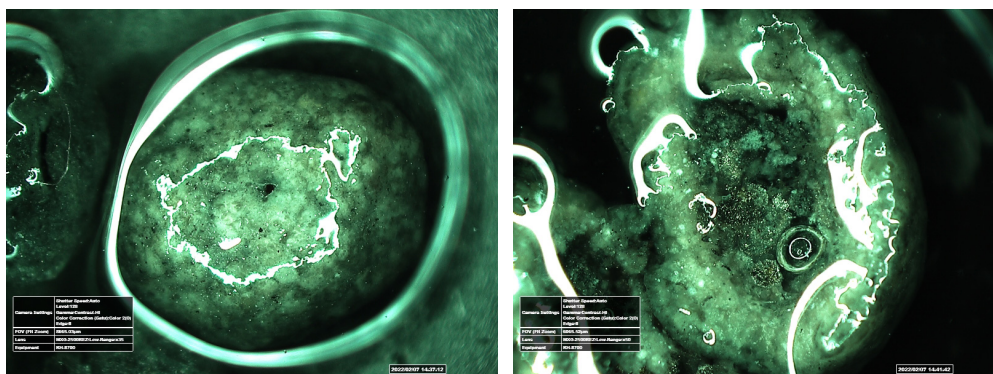


Figure 1. Visualization of raw anaerobic granular sludge at 35 × magnification

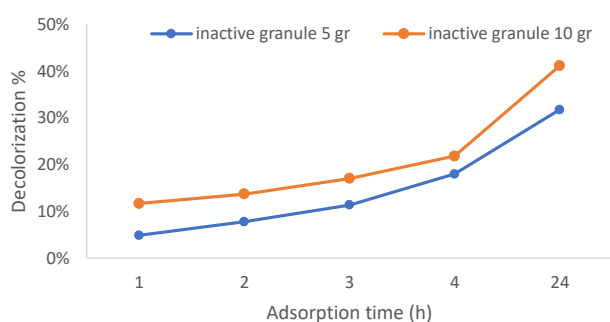


Figure 2. Adsorption capacity of inactive granules

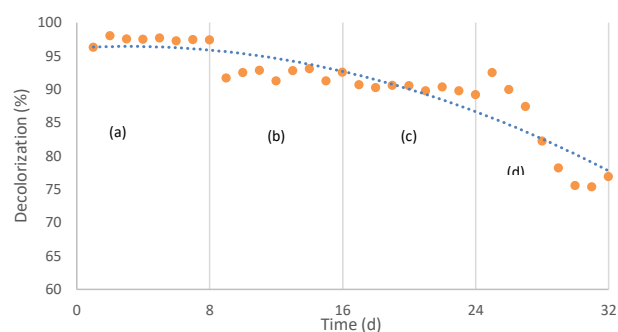


Figure 4. Decolorization rate (%) at (a) 50 mg/L, (b) 100 mg/L, and (c) 150 mg/L dye

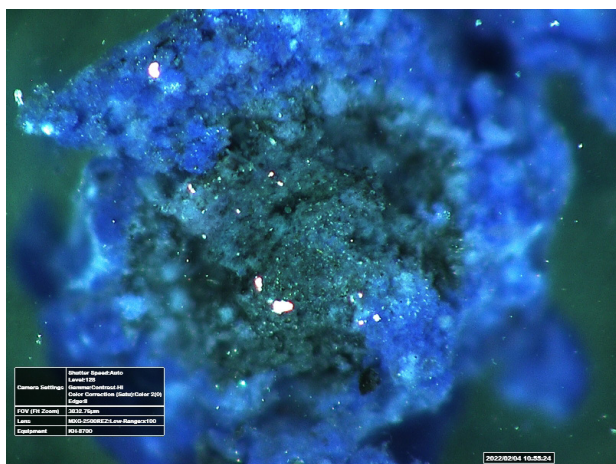


Figure 3. Visualization of inactive granule after experiment at 100 × magnification

evaluating effluent toxicity.

Stability of ACoD system

The VFA/TA ratio was selected as an indicator for system stability because alkalinity may serve as a tool to neutralize the VFA generated and prevent pH drop. Decreased alkalinity may indicate a disturbance in the system. Additionally, TA and VFA were monitored to evaluate the stability of the anaerobic digester. Increasing the dye concentration to 100 mg/L affected the ACoD system significantly (Figure 7). Alkalinity decreased significantly from 874.44 mg/L to 1369.43 mg/L at a dye concentration

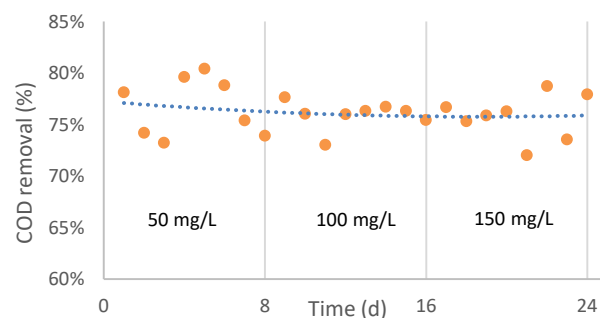


Figure 5. COD removal at dye concentrations of 50 mg/L, 100 mg/L, and 150 mg/L

Table 2. Dye degradation at different inlet concentrations

Initial dye concentration (mg/L)	Decolorization (%)	Dye degradation (mg/day)
50	97.37	233.68
100	92.22	442.65
150	90.12	648.86

of 50 mg/L and 635.72 mg/L–827.28 mg/L at 100 mg/L. Then, it slightly increased at a dye concentration of 150 mg/L in the range of 730.92 mg/L–949.57 mg/L. Conversely, a relatively stable VFA at a dye concentration of 100 mg/L was observed, ranging from 58.68 mg/L to 114.35 mg/L, indicating well-balanced VFA generation and utilization. At a dye concentration of 150 mg/L, VFA concentration increased significantly.

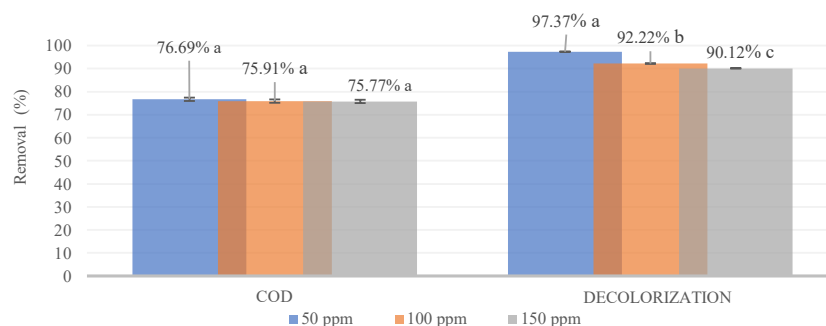


Figure 6. COD removal and decolorization trends at different dye concentrations

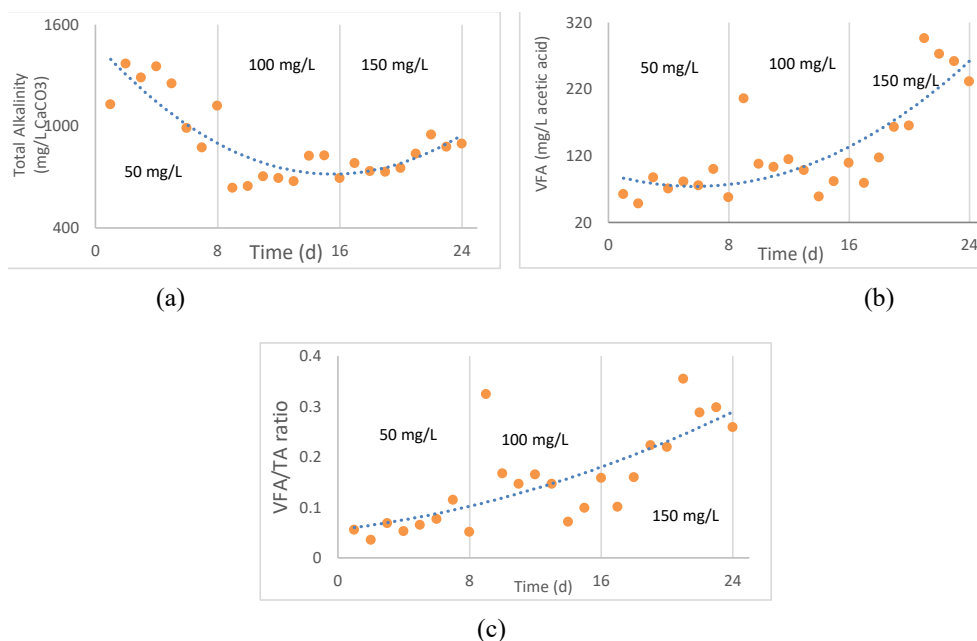


Figure 7. The trend of (a) total alkalinity (TA), (b) volatile fatty acid (VFA), and (c) VFA/TA ratio at dye concentrations of 50 mg/L, 100 mg/L, and 150 mg/L.

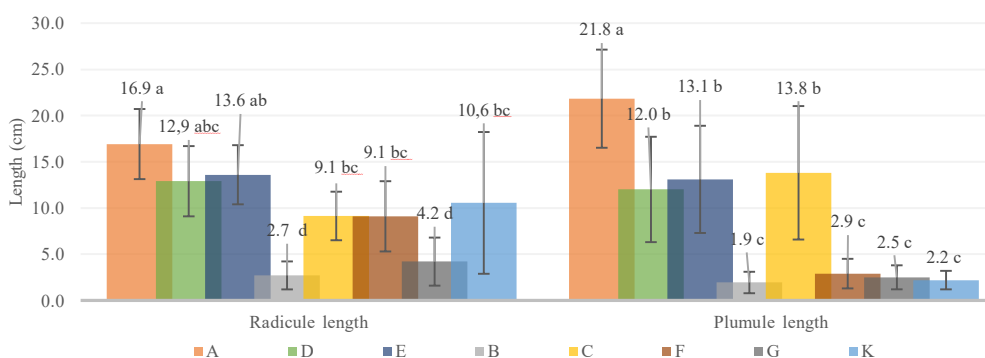


Figure 8. Phytotoxicity test based on radicle and plumule growth (n = 15) of (A) 50 mg/L ACoD effluent, (B) synthetic wastewater 50 mg/L, (C) dye 50 mg/L, (D) 100 mg/L ACoD effluent, (E) 150 mg/L ACoD effluent, (F), dye 100 mg/L, (G) dye 150 mg/L, and (K) control/distilled water

A VFA/TA ratio of 0.23–0.3 represents stable AD (24), whereas a ratio of 0.25–0.35 indicates a healthy anaerobic system (25). The ACoD system was slightly disturbed due to an increased dye concentration (Figure 7). The VFA/TA ratio spiked markedly from 0.05 to 0.32 from the beginning of an increase in dye concentration to 100 mg/L, and then stabilized in the range of 0.07–0.16.

Phytotoxicity test

A phytotoxicity test was used to evaluate the toxicity level of ACoD effluents. The longest radicle of 16.9 cm was observed at an effluent concentration of 50 mg/L, whereas at dye concentrations of 100 mg/L and 150 mg/L, radicle lengths were 12.9 cm and 13.6 cm, respectively (Figure 8). At the inlet dye concentrations of 50 and 100

mg/L, the radicle length was slightly lower than that in the control group (distilled water), but the difference was not statistically significant ($P < 0.05$).

Germination studies have been performed to evaluate dye toxicity. GI has been specifically used to determine phytotoxicity due to its high sensitivity (20). The phytotoxicity test showed that AcoD effluent dye concentrations of 50 mg/L and 100 mg/L caused 14.15% inhibition, whereas an AcoD effluent dye concentration of 150 mg/L caused 60.38% inhibition. Additionally, raw dye wastewater caused 74.3% inhibition.

Discussion

Characterization of anaerobic granules

Granule is the main component in granular sludge reactors for biological wastewater treatment to improve pollutant removal efficiency. Anaerobic granules are commonly characterized by biogas-releasing craters on their surface. During the granulation process, microbubbles generated inside granular sludge converge to the main gas channel, forming observable gas bubbles (26). The greyish-white and black colors observed on the outer layer and in the center of the granule may indicate the predominance of fermentative or hydrolytic bacteria in the outer granular layer and that of acetoclastic methanogenic bacteria, which are essential for the removal and degradation of pollutants, in the center portion (Figure 1). Notably, the degradation of organic matter is initiated by the hydrolysis of complex compounds, such as polysaccharides, proteins, and lipids, into simpler intermediate compounds, which are water-soluble and absorbed into granules. These organic and acetic acids are then utilized by methanogenic bacteria located at the center of the granule to generate methane. This observation is consistent with the findings of previous studies, showing the importance of this layered structure in creating a healthy syntrophic relationship among microbes (27). This specific shape can facilitate inter-species electron transport, a process essential for bacterial metabolism (28).

Bacterial adsorption efficiency of the granular sludge

Higher decolorization performance at 10 gr granule was attributed to the higher amount of granule used (Figure 2). This result is consistent with the findings of previous studies reporting a relatively low decolorization capacity of inactive granules, with only < 10 mg/g (29) and 26 mg/g methylene blue (30). The dye-mediated saturation of granules quickly limits bacterial adsorption, leading to a gradually decreasing decolorization performance. However, bacterial activity-induced reductive decolorization might be slower than bacterial adsorption, but it is more reliable as it occurs in a long-term process.

Figure 3 suggests that the adsorption mechanism will occur initially, providing an advantage for the degradation process. Pollutants will be adsorbed into the

granule, enhancing the contact with microorganisms and improving removal efficiency, including decolorization. Furthermore, it indicates that reductive decolorization occurs after the adsorption mechanism.

Decolorization performance of the AcoD system

In AD, decolorization is associated with two mechanisms, namely, reductive decolorization and bacterial adsorption, which occur simultaneously but with different orders of magnitude. Bacterial adsorption occurs promptly and quickly at the beginning of the process, as the dye is adsorbed, mainly on the surface and center of the granule (Figure 3). Reductive decolorization occurs because of the cracking of conjugated dye bonds by reductase (31).

Herein, decolorization did not completely depend on enzymatic decolorization but on reducing equivalents (electrons) released by bacterial metabolism (32). Reducing equivalents from honey processing wastewater were transferred to Remazol blue RSP as electron donors, causing the bond cleavage and shift in the chemical structure of the dye, ultimately leading to decolorization. The notable and gradual decrease in decolorization observed in the mono-digestion system further supported these results. In the monodigestion system, with the absence of honey processing wastewater as the primary electron donor, decolorization was substantially decreased from 92.5% to 76.89% at the end of the experiment (Figure 4).

Lower dye degradation in the AcoD system compared to the previously reported result (23) was attributed to the different types of granules and dyes used in the present study (Table 2). It could be caused by the limited capacity of microorganisms used and the increased toxicity caused by higher dye concentrations. Yadav et al reported an optimized decolorization of Rapid Remazol Brilliant Blue dye (97.18%) using laccase (33). This is possibly attributed to the lower dye concentration used in the study compared to the present work (100 mg/L). Dokpikul et al. combined UV irradiation and electrooxidation process (UV/EO) and achieved high decolorization (99%) of Methylene Blue (MB) after 60 min (34). This is likely caused by the sensitivity of MB to the free radicals ($\text{HO}^\bullet$ and $\text{Cl}^\bullet$) generated by the electrooxidation process.

The absorbance peaks at 595 nm disappeared irreversibly after AcoD, indicating the effectiveness of AcoD in dye decolorization (Figure S3). No new peak was observed in the visible spectrum of AcoD effluents, indicating that the anthraquinone chromophore was completely decolorized without forming new colors. This result is consistent with the results of previous studies on the decolorization of diverse dyes and textile wastewater. For instance, 90% decolorization of Remazol brilliant blue R using *Enterobacter* sp. strain F, 73% decolorization of textile wastewater using an endogenous anaerobic consortium, and 68% decolorization of the direct black

dye have been previously reported (35–37).

COD removal efficiency of the ACoD system

Relatively stable COD removal (620.85 ± 19.38 , 679.57 ± 2.41 , and 627.72 ± 10.13 mg/L) was attributed mainly to almost similar levels of COD influent at dye concentrations of 50, 100, and 150 mg/L, respectively (Figure 5). Relatively high dye degradation was attributed to the in-depth wastewater degradation to the core of the granule, in which wastewater could penetrate and enter the center of the granule, which is the location of hydrolytic-fermentative and acidogenic-acetogenic colonies. Thus, contact between pollutants and bacteria was beyond the surface, which resulted in higher degradation performance. The COD removal rate achieved in this study was relatively higher than the findings reported by Lourenço et al. The authors achieved COD removal of 55–77% in the textile wastewater treatment containing azo dye using aerobic granular sludge (AGS) (38). This is attributed to the susceptibility of the AGS system in the presence of dye and COD shock loads.

In the present ACoD system, the dye and honey wastewater were degraded simultaneously and complementarily. Owing to the roles of the dye as an electron acceptor and honey wastewater as an electron donor, processing them together was more advantageous than treating them separately. As an electron acceptor, the dye prevented VFA accumulation or overload in high-strength organic wastewater treatment (7), whereas honey wastewater increased decolorization and bond cleavage of the dye. However, despite being low in COD, wastewater with high dye concentrations affected the ACoD system owing to the toxic characteristics of the dye. At the end of the experiment, the effect was evident from the signs of unstable COD removal and an increasing VFA/TA ratio at a dye concentration of 150 mg/L. The reason was an unequal amount of honey wastewater as a co-substrate compared to an increasing dye concentration. Thus, an appropriate ratio of dye and honey wastewater is necessary to ensure a stable and high-performance of the ACoD system.

The bacterial degradation of dye involves the dissociation of small molecular groups around anthraquinone rings (39), which form intermediates such as sodium 1-amino-9,10-dioxo-9,10-dihydroanthracene-2 sulfonate and sodium 2-((3-aminophenyl)sulfonyl)ethyl sulfate (40) (Figure S4). The anthraquinone ring is gradually decomposed via oxidation and hydrolysis, resulting in simpler compounds. Different intermediates have been reported in the bacterial degradation of dye, which depend on functional groups. However, bacteria generally exhibit similar metabolic pathways, including demethylation, hydrogenation reduction, dehydroxylation, and other biochemical processes.

Trend of COD removal and decolorization rate

Increasing dye concentrations did not notably affect COD removal (Figure 6). Similarly, another study reported that RBBR concentrations up to 150 mg/L did not disrupt COD removal performance (41). The organic loading rate was the limiting factor for COD removal in anaerobic digestion systems at ≤ 150 mg/L dye concentrations. The average decolorization performances at concentrations of 50, 100, and 150 mg/L were 97.37%, 92.22%, and 90.12%, respectively, which were significantly different ($P > 0.05$), indicating that higher dye concentrations resulted in lower color removal. Despite lower color removal, dye degradation was higher when the ACoD system was used.

Effect of dye concentrations on ACoD system stability

Initially, a relatively low VFA concentration of 78.73 mg/L indicated the beginning of adaptation to the increased dye concentration. However, VFA concentration then markedly increased on the fifth day (296.63 mg/L) and continued to decrease (231.55 mg/L) on the last day of the experiment (Figure 7). An increased VFA concentration was not accompanied by improved buffering capacity, thus leading to a higher VFA/TA ratio (> 0.2) (Figure 7c). A VFA/TA ratio of 0.23–0.3 indicates stable AD, < 0.23 indicates an underfed system, and > 0.3 indicates a poor stability of AD (24). This phenomenon could be attributed to VFA accumulation in the system. Long-chain fatty acids generated during dye degradation are not readily used by methanogenic microorganisms and are converted into methane; thus, they may inhibit the activities of methanogenic microorganisms and result in VFA accumulation (42,43). Another reason behind the increased VFA concentration observed was the effect of higher dye concentrations, which could potentially inhibit the activity of acetoclastic methanogens. Acetoclastic methanogens are commonly sensitive to high dye concentrations (44).

A stable VFA/TA ratio (0.07–0.16) at a dye concentration of 100 mg/L indicated a healthy anaerobic co-digestion system (Figure 7c). In other words, the ACoD system was readily adapted to the increased dye concentration, though the ratio was relatively higher than that observed at a dye concentration of 50 mg/L. An opposite trend was observed at a dye concentration of 150 mg/L, wherein the VFA/TA ratio increased gradually and remained at a maximum of 0.35, indicating the potentially unstable ACoD system. A relatively increased VFA/TA ratio at a dye concentration of 150 mg/L was attributed to an increased VFA concentration, which was not followed by increased alkalinity. This indicated low bacterial activity during dye degradation owing to an insufficient organic co-substrate in dye wastewater and increased toxic substances from the dye, because methanogen activity was highly likely affected by the dye concentration. In the long term, this would lead to system instability and shock.

Phytotoxicity test

Radicule lengths at dye concentrations of 100 mg/L and 150 mg/L were observed as not statistically different ($P < 0.05$) (Figure 8), indicating that ACoD effluents can be used to support the growth of seedlings. However, the length differed significantly when an inlet dye concentration of 150 mg/L was used. The average radicle length was 4.2 cm, indicating high dye toxicity at 150 mg/L, which affected radicle growth. This result is consistent with the trend observed by Dhaouefi et al who reported 52% inhibition of *Raphanus sativus* growth when grown in dispersed dye wastewater (45).

The phytotoxicity results were consistent with the GI, which revealed that ACoD effluent dye concentrations of 50 mg/L and 100 mg/L did not primarily inhibit seed germination, indicating a GI of 85.85%. In contrast, an increased dye concentration of 150 mg/L significantly reduced GI to 39.62%, slightly higher than the GI of the raw inlet (25.47%). Additionally, plumule measurements showed a similar trend. The longest plumule of 21.8 was observed at a dye concentration of 50 mg/L, whereas 12 cm and 13.1 cm were observed at 100 mg/L and 150 mg/L, respectively. These observations supported the above-mentioned statement that the stability of the ACoD system was highly affected by an increased dye concentration of 150 mg/L owing to highly toxic dye wastewater. The phytotoxicity test results suggested that the ACoD system could significantly reduce dye wastewater toxicity.

Conclusion

The presence of dye in textile wastewater may pose severe risks to aquatic ecosystems and human health. This study proposed an anaerobic co-digestion system for treating synthetic textile wastewater containing Remazol blue RSP using honey wastewater as a co-substrate. The effect of dye concentrations on the performance of the ACoD system was investigated and evaluated. The findings showed that the decolorization capacity of the ACoD system was significantly reduced at higher concentrations, indicating the effect of dye concentrations on the decolorization rate. Similarly, system instability was observed at higher dye concentrations, reflecting that dye concentrations highly affect the anaerobic system, possibly attributed to the inhibition of acetoclastic methanogens. In contrast, the system showed relatively similar COD removal at all dye concentrations, reflecting that dye concentration did not influence the overall pollutant removal. The decolorization capacity also decreased significantly in the absence of honey processing wastewater (mono-digestion system), highlighting the role of co-substrate as the electron donor. Finally, phytotoxicity tests showed that ACoD significantly improved the quality of dye wastewater and reduced dye toxicity.

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Formal analysis: Hanny Vistanty.

Funding acquisition: Rizal Awaludin Malik.

Investigation: Hanny Vistanty, Rizal Awaludin Malik.

Methodology: Rizal Awaludin Malik.

Project administration: Rizal Awaludin Malik.

Resources: Rizal Awaludin Malik.

Supervision: Sri Harjati Suhardi.

Validation: Sri Harjati Suhardi.

Visualization: Hanny Vistanty.

Writing – original draft: Hanny Vistanty.

Writing – review & editing: Hanny Vistanty, Rizal Awaludin Malik, Sri Harjati Suhardi.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues

The authors state that some of the data collected during the research are presented in the manuscript, and several parts have been published elsewhere separately, each with its own perspective and discussion. The data are mutually supportive.

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Supplementary file

Supplementary file contains Figure S1- S4, Table S1, and S2.

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