

©Western Philippines University  
 ISSN: 1656-4707  
 E-ISSN: 2467-5903  
 Homepage: [www.palawanscientist.org](http://www.palawanscientist.org)

# Design of a compact wastewater treatment and isolation of microbial consortia for nutrient reduction of plastic recycling wastewater

Jaren U. Tulipan and Jey-R S. Ventura\*

*Biomaterials and Environmental Engineering Laboratory, Department of Engineering Science, College of Engineering and Agro-Industrial Technology, University of the Philippines Los Baños, College, Laguna, Philippines*

\*Correspondence: [jsventura@up.edu.ph](mailto:jsventura@up.edu.ph)

Received: 31 May 2023 || Revised: 03 Oct. 2023 || Accepted: 19 Oct. 2023  
<https://doi.org/10.69721/TPS.J.2023.15.2.03>

## How to cite:

Tulipan JU and Ventura JS. 2023. Design of a compact wastewater treatment and isolation of microbial consortia for nutrient reduction of plastic recycling wastewater. *The Palawan Scientist*, 15 (2): 21-30.  
<https://doi.org/10.69721/TPS.J.2023.15.2.03>

## ABSTRACT

The wastewater generated during the plastic recycling is an aspect that is often overlooked in the process. This wastewater contains pollutants that can affect the environment and human health. To address this problem, a study was conducted to design and evaluate the performance of an anoxic-oxic (AO) system and an anoxic-oxic-oxic (AO<sub>2</sub>) system in treating plastic recycling wastewater. Additionally, the study collected wastewater from a plastic recycling company and activated sludge from a sewage treatment plant to test the viability of biological treatment for treating plastic recycling wastewater. The results showed that both systems were effective at reducing chemical oxygen demand (COD), with the AO system having a higher percent COD removal (98.13%) than the AO<sub>2</sub> system (85.33%). Also, there were 18 unique colonies isolated from the wastewater and activated sludge. Overall, the study concludes that biological treatment can be used to treat plastic recycling wastewater, and the design of upscaled system is crucial to fully solve the wastewater problem.

**Keywords:** laboratory scale reactor, recycling industry wastewater, wastewater treatment design

## INTRODUCTION

Plastic waste has become a major environmental issue in recent years, with much attention focused on the impact of plastic pollution on the environment and human health. One of the most effective ways to reduce the environmental impact of plastic waste is to recycle it. Plastic recycling is the process of transforming discarded plastic materials into new products that can be used again (Hopewell et al. 2009). However, one aspect of the problem that is often overlooked is the wastewater generated during the plastic recycling process (Gunarathna et al. 2010).

The washing stage plays a pivotal role in removing residues such as wood, pulp fibers, food, and adhesives, which otherwise would have detrimental effects on the final regenerated product. Therefore, these washing stages produce vast amounts of wastewater. The current washing technology used by plastic recyclers has significantly reduced water consumption to 2-3 m<sup>3</sup> of water per ton of plastic material (Altieri et al. 2021). This wastewater can contain a variety of hazardous chemicals and contaminants, and if not properly treated, it can have serious negative impacts on the environment and human health (Setiawan et al. 2021).



This article is licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](https://creativecommons.org/licenses/by-nc/4.0/)

The composition of wastewater varies depending on the source. Altieri et al. (2021) investigated plastic solid waste washing wastewater and found that it contains high amounts of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total nitrogen (TN). The high concentration of these pollutants may be attributed to the source of the plastic solid waste, which was mainly from the agro-industrial sector. Setiawan et al. (2021) characterized polyester synthetic fiber industry wastewater and found that it contains high amounts of COD, BOD, and total suspended solids (TSS). Santos et al. (2005) characterized effluents from the washing steps of polyethylene terephthalate (PET) and polyolefins cleaning process, while Santhmayor et al. (2020) characterized wastewater from waste plastic recycling machines. Both studies indicated high amounts of COD and TSS in the wastewater. The high COD values may be attributed to the surfactant used in the washing step of the plastics (Santos et al. 2005). Meanwhile, Kolbl (2016) characterized plastic processing plant wastewater and found a high amount of total phosphorous (TP). The above findings show that plastic recycling wastewater can have high amounts of COD, BOD, TP, TN, and TSS. High levels of these pollutants may cause nitrate contamination, eutrophication, and ammonia toxicity (Curtin et al. 2011). Additionally, high levels of TSS may cause problems for aquatic life as TSS may include solids from the plastic recycling wastewater that can harm the environment (Santhmayor et al. 2020). These results show proof that plastic recycling wastewater can be harmful. Therefore, proper treatment of plastic recycling wastewater is essential to prevent these negative impacts.

Plastic recycling wastewater poses harmful effects on the environment, and there is still a lack of available and cost-effective technology for the plastic recycling industry to implement. In the case of Valenzuela City, Philippines, the researchers found that most plastic recycling facilities do not have the necessary equipment or infrastructure to properly treat wastewater. Most of the facilities reuse their wastewater without treatment to cut operating costs. This is not only hazardous but also decreases the quality of recycled plastic (Altieri et al. 2021). Efforts to mitigate the effects of this in the wastewater were made by applying flocculation methods. However, the lack of knowledge on wastewater composition and appropriate treatment system makes it difficult to assess.

To address this problem, this study aimed to design and evaluate the performance of an anoxic-oxic (AO) system and an anoxic-oxic-oxic (AO<sub>2</sub>) system in treating plastic recycling wastewater. These systems were investigated for their potential to enhance biological nutrient removal from plastic recycling wastewater. Also, developing these systems ensures a

low-cost technology that can be adapted by the plastic recycling industry. The specific objectives were the following: (1) to characterize the wastewater generated by a local plastic recycling facility in Valenzuela City, Philippines; (2) to isolate and assess the potential of microbial consortia found in activated sludge for the purpose of treating plastic recycling wastewater; and (3) to determine the removal efficiencies and COD reduction performance of the AO and AO<sub>2</sub> systems. Through these objectives, the study sought to contribute valuable insights into the treatment of plastic recycling wastewater.

## METHODS

### Wastewater Collection and Characterization

Twenty liters of wastewater from a plastic recycling company in Valenzuela City, Philippines were collected and stored. The wastewater was subjected to analysis using standard methods for the examination of water and wastewater to determine the following parameters: COD, BOD, nitrates, phosphates, TSS, pH, and dissolved oxygen.

### Sludge Collection and Acclimatization

Similarly, 20-L of activated sludge were collected from a sewage treatment plant in Biñan, Laguna, Philippines, and divided into two setups for acclimatization in synthetic wastewater. One setup was anoxic, while the other was aerobic using an aquarium pump for aeration. The synthetic wastewater used is composed of 5 g L<sup>-1</sup> glucose, 1 g L<sup>-1</sup> NH<sub>4</sub>Cl, 1 g L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub>, 1 g L<sup>-1</sup> NaHCO<sub>3</sub>, 0.2 g L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, and 1 mL L<sup>-1</sup> of trace metal solution (Alzate-Gavira et al. 2003). To maintain the culture setups, synthetic wastewater was regularly replaced.

### Isolation of Bacteria from the Activated sludge

**Microbial preservation and isolation.** To have a starting culture for the wastewater treatment facility, the microbial consortia from the activated sludge were isolated and stored. Ten mL samples were taken from the treated wastewater, the mixed liquor from the anoxic setup, and the settled activated sludge solution. The samples were stored in 2-mL centrifuge tubes. Then, serial dilution of each sample was done up to 10<sup>-5</sup> dilutions. Plate count agar (PCA) was prepared to serve as the growth medium for the microorganisms in the activated sludge because it is usually used for bacterial enumeration in water and wastewater (Aryal 2022; Ogado et al. 2022). It is composed of 5 g L<sup>-1</sup> casein hydrolysate, 2.5 g L<sup>-1</sup> yeast extract, 1 g L<sup>-1</sup> glucose, and 15 g L<sup>-1</sup> agar.

After which, the pour plate method was then used. Four plates of treated wastewater, an anoxic setup of mixed liquor, and an activated sludge solution were made. Then, two plates of each were placed in a

desiccator to create an anaerobic condition. The other two plates of each were placed in storage to facilitate aerobic conditions. Under these conditions, the microorganisms were allowed to grow for two days.

Luria Bertani (LB) broth was prepared to serve as the medium for the growth cultivation of bacteria found in the plates. It is composed of  $100 \text{ g L}^{-1}$  casein hydrolysate,  $50 \text{ g L}^{-1}$  yeast extract, and  $60 \text{ g L}^{-1}$  glucose. The bacteria colonies found in each plate were placed on test tubes filled with 15 mL of LB broth. Two test tubes of colonies found from treated wastewater, anoxic setup mixed liquor, and activated sludge solution were placed in a desiccator to facilitate anaerobic conditions. The other two test tubes were placed in storage to facilitate aerobic conditions. The bacteria were allowed to grow for three days. After which, glycerol stocks were made from each test tube. The isolates were then stored at  $4^\circ\text{C}$  with 1 mL of LB broth and 1 mL of glycerol.

**Regrowing and characterization of the stock inoculum.** Plate count agar with nystatin ( $10,000 \text{ units ml}^{-1}$ ) was prepared and served as a medium for growth for the microbial consortia plates. Nystatin was added to ensure that only bacteria would grow on the PCA plates. A total of 24 plates of PCA were prepared. Four plates were prepared for both anaerobic and aerobic setups for treated wastewater, anoxic setup mixed liquor, and activated sludge solution. The plates were incubated in an anaerobic (using a desiccator) and aerobic condition for approximately 4 days to allow the bacteria to grow.

Colonies were identified based on their observed characteristics on the plates. Gram staining was done to further characterize the unique bacteria colonies by their cell membrane structures. The gram-stained bacteria were observed under a microscope.

### AO and AO<sub>2</sub> Reactor Design and Fabrication

**AO system.** A laboratory-scale AO system was designed to determine the COD reduction of the system using the activated sludge. Design considerations were as follows. The volume of the oxic chamber and the anoxic chamber was determined in the ratio of 1:2. The height of the reactor was set to 120 mm, which is roughly the same height as a 250-mL beaker. The width of the reactor was set to 68 mm to fit a magnetic stirrer inside the reactor. To achieve a 1:2 volume ratio for the oxic and anoxic chambers, the length of each chamber was set to 68 mm and 136 mm, respectively. Alternating hanging and standing baffles were placed inside the reactor to compartmentalize the reactors and regulate the flow of liquid from the anoxic chamber to the oxic chamber. The baffles are 3 mm thick and have vertical clearances of 20 mm. The reactor was fabricated by 3D printing using Creality CR-10 Max. The 3D printing parameters were based

on the study of Nasr Esfahani et al. (2021) using polylactic acid (PLA) filament for the printing process. The wall thickness of the reactor was set to 4 mm, and the base thickness was set to 10 mm. The infill pattern was set to tri-hexagon with an infill density of 75% (Nasr Esfahani et al. 2021). An outlet was placed at the same height as the standing baffle. The 3D model of the fabricated AO reactor is shown in Figure 1.

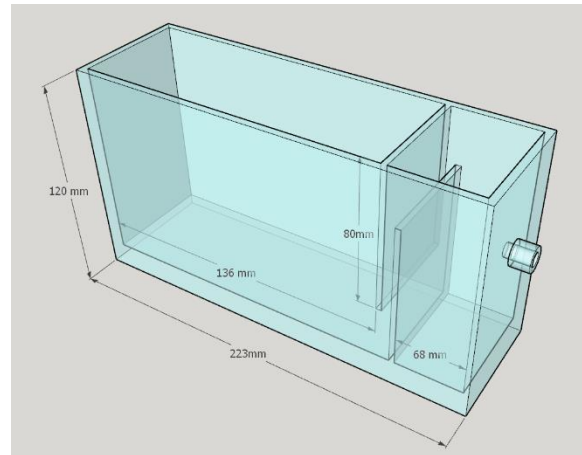


Figure 1. 3D model of the fabricated anoxic-oxic reactor.

The AO system configuration is depicted in Figure 2. The hydraulic retention time (HRT) for the anoxic chamber was set to 16 hr and the HRT for the oxic chamber was set to 8 hr. Meanwhile, the pH and the temperature in both the anoxic and oxic chambers were set at pH 7 and  $25^\circ\text{C}$ . Meanwhile, the influent flowrate and the effluent flowrate were both set at  $1 \text{ m}^3 \text{ d}^{-1}$ .

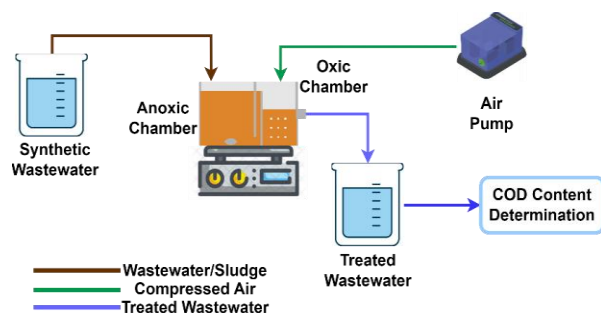
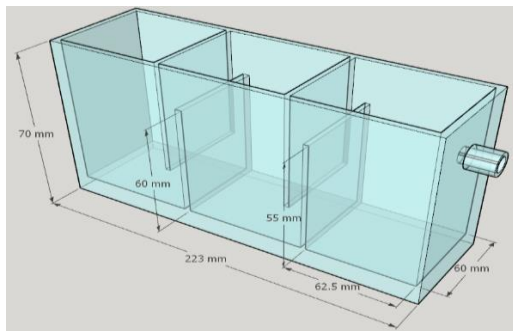


Figure 2. Anoxic-oxic system configuration.

**AO<sub>2</sub> system.** From the results of the AO laboratory scale reactor, it was determined to improve the system configuration by adding another oxic chamber to the design. With this, an AO<sub>2</sub> laboratory scale reactor with cascading baffles was designed. The height of the reactor was set to 70 mm. The wall thickness of the reactor was set to 4 mm, while the base thickness was set to 5 mm. The reactor consists of three chambers – one anoxic chamber and two oxic chambers. The chambers were equal in area, with

length and width measuring 62.5 mm and 60 mm, respectively. Alternating hanging and standing baffles were designed to compartmentalize the chambers and regulate the wastewater flow. The standing baffles were designed to have a 5 mm decrease in height to facilitate cascading flow from one chamber to the next. The standing baffle heights were 60 mm and 55 mm with a vertical clearance of 20 mm. A single outlet was placed at the same height as the last standing baffle and has an inner diameter of 10 mm.

A 3D printed laboratory scale reactor was used in the setup. The infill design was chosen to be tri-hexagon to have better 2D stress management. The infill density was set to be 60%, which was found to be sturdy enough to prevent any leakages based on previous test prints. The 3D model of the fabricated AO<sub>2</sub> reactor is shown in Figure 3. The AO<sub>2</sub> system configuration is shown in Figure 4. The hydraulic retention time (HRT) for the anoxic and two oxic chambers was set to 8 hr. The pH and the temperature in all the chambers were set at pH 7 and 25°C, respectively. Meanwhile, the influent flowrate and the effluent flowrate were both set at 1 m<sup>3</sup> d<sup>-1</sup>.



**Figure 3.** 3D model of the fabricated anoxic-oxic-oxic reactor.

**Chemical Oxygen Demand Reduction Performance**

A10-L container containing synthetic wastewater served as the influent into the system. The wastewater was continuously fed to the reactors at a



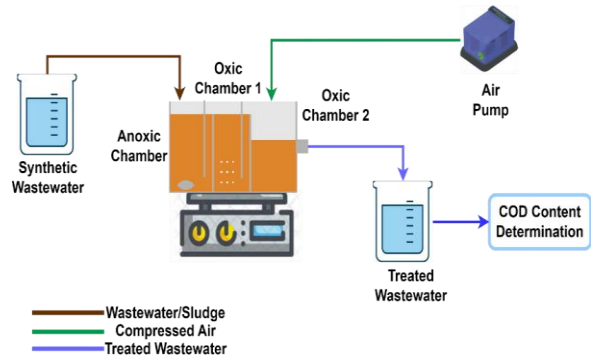
$$\text{Theoretical COD} = \text{mol oxidizable material} \times \frac{\text{mol } O_2}{\text{mol oxidizable material}} \times \text{MM } O_2 \tag{Equation 2}$$

$$\text{Absorbance (nm)} = 0.1396 + 0.5827 * \text{COD (g L}^{-1}\text{)} \tag{Equation 3}$$

$$\text{COD reduction rate} = \frac{\text{Initial COD concentration} - \text{Final COD Concentration}}{\text{Final time} - \text{initial time}} \tag{Equation 4}$$

$$\% \text{ COD reduction} = \frac{\text{Initial COD concentration} - \text{Final COD Concentration}}{\text{Initial COD Concentration}} \times 100 \tag{Equation 5}$$

rate of 1 L day<sup>-1</sup>. The collected activated sludge was placed in the different chambers of the reactor. An air stone was placed in the oxic chamber that was connected to an air pump, which served as the air supply for the oxic chamber, while a magnetic stirrer was placed inside the anoxic chamber.



**Figure 4.** Anoxic-oxic-oxic system configuration.

Meanwhile, the COD of the influent and effluent were measured using the dinitrosalicylic acid (DNS) method, which is based on the National Renewable Energy Laboratory's method for measuring cellulase activity (Adney and Baker 2008). The DNS method was used to determine the COD reduction performance of the reactors by assuming that the glucose content measured by the DNS method is equal to the COD content of the samples. The theoretical COD can be calculated based on the combustion reaction of glucose and oxygen to produce carbon dioxide and water, as seen in Equation 1. Then the theoretical COD can be calculated using Equation 2. Using Equation 2, it can be calculated that for 1000 ppm of glucose, the COD is approximately 1067 ppm. Equation 3 shows the standard curve equation used to determine the COD of the samples for this experiment. The COD reduction rate and percent COD reduction were calculated using Equations 4 and 5.

The reagent for the DNS method was prepared by adding the following chemicals to 1 L of distilled water: 7.5 g dinitrosalicylic acid, 14 g NaOH, 216 g  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ , 5.4 mL phenol, and 5.9 g  $\text{Na}_2\text{S}_2\text{O}_5$ . A 1.5 mL of this reagent was then mixed with 0.75 mL of wastewater. The mixture was then heated at 100°C for 5 min and cooled in an ice water bath to develop color. The absorbance of this color was then measured using a spectrophotometer at 540 nm and compared to a standard curve to determine the COD concentration in the wastewater. The standard curve was generated by making standard solutions of glucose with concentrations of 0.2 g L<sup>-1</sup> to 1.2 g L<sup>-1</sup>.

## RESULTS

### Plastic Recycling Wastewater Characteristics

The collected wastewater from the plastic recycling industry was subjected to wastewater characteristic analysis using standard methods. The results are shown in Table 1. From the table, the BOD, nitrate, phosphate and TSS concentration in the wastewater were 651 mg L<sup>-1</sup>, 337 mg L<sup>-1</sup>, 3.16 mg L<sup>-1</sup>, and 338 mg L<sup>-1</sup> respectively. Meanwhile, the COD concentration of the wastewater was 5682 mg L<sup>-1</sup>. Fecal coliform was also found in the wastewater at 1.8 MPN per 100 mL. The color of the wastewater was

measured at 25 TCU. The pH and temperature of the wastewater were 6.47 and 28.2 °C, respectively.

### Bacterial Isolates from Activated Sludge

Tables 2 and 3 summarize the colony and bacterial characteristics observed in the isolates. In total, 18 unique isolates were observed. Seven aerobic isolates were identified from the PCA plates while eleven anaerobic isolates were identified from the PCA plates.

### Performance of the AO and AO<sub>2</sub> System

The percent COD reduction and the COD reduction rate of the AO system are shown in Figure 5. The figure shows that on the first day, there was a COD reduction rate of 60.42 mg L<sup>-1</sup> h<sup>-1</sup>, which translates to a 99.01% reduction. This high reduction rate was maintained throughout the process. Overall, the process was highly effective, with all reduction rates above 92%. Meanwhile, the percent COD reduction and the COD reduction rate of the AO<sub>2</sub> system are shown in Figure 6. The figure shows that the system was stable at the start of the experiments but was unstable towards the end. The AO system showed a more stable treatment efficiency as compared to the AO<sub>2</sub> system in terms of COD reduction performance.

**Table 1.** Wastewater characteristics of plastic recycling industry effluent as compared to DENR general effluent standards. SM – standard methods for the examination of water and wastewater.

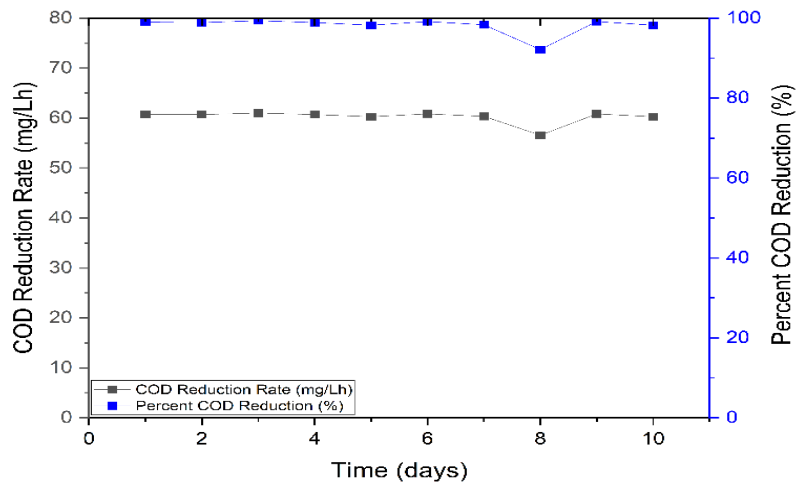
Analysis	Method	Unit	Wastewater	DENR GES Class C
Fecal Coliform	SM 9221 E	MPN per100 mL	1.8	400
Color	SM 2120 B	TCU	25	150
Nitrate	SM 4500 – NO <sub>3</sub> E.	mg L <sup>-1</sup>	337	14
COD	SM 5220 B	mg L <sup>-1</sup>	5682	100
BOD	SM 5210 B.	mg L <sup>-1</sup>	651	50
TSS	SN 2540 D.	mg L <sup>-1</sup>	338	100
Phosphate	SN 4500-P D.	mg L <sup>-1</sup>	3.16	4
pH	-	pH	6.47	6.0-9.5
Temperature	-	°C	28.2	25-31

**Table 2.** Colony and bacterial characteristics of isolates from aerobic conditions.

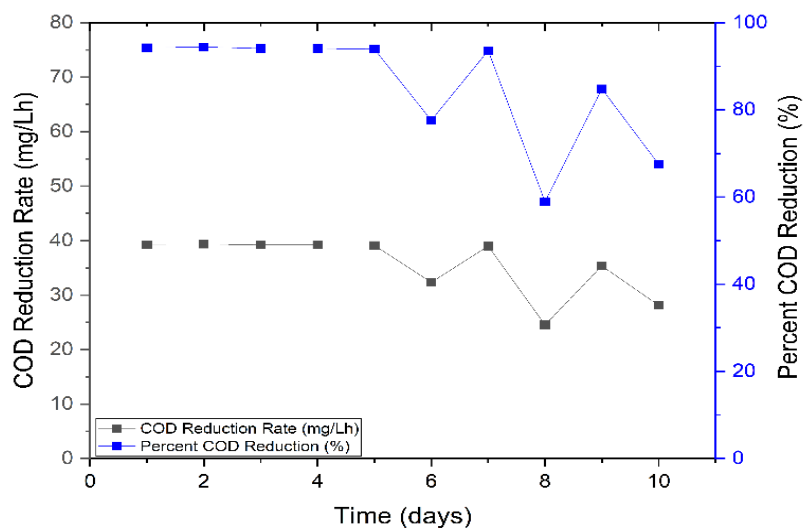
Isolate	Colony Characteristics			Bacterial Characteristics	
	Color	Form	Elevation	Gram Stain	Shape
WW O <sub>2</sub> 1	Creamy	Circular	Raised	Gram-negative	Rod
WW O <sub>2</sub> 2	White	Circular	Flat	Gram-negative	Rod
WW O <sub>2</sub> 3	Bright Orange	Circular	Raised	Gram-negative	Rod
WW O <sub>2</sub> 4	Whitish Yellow	Circular	Flat	Gram-positive	Rod
Sol'n O <sub>2</sub> 1	Creamy	Irregular	Flat	Gram-negative	Rod
Sol'n O <sub>2</sub> 2	Whitish Yellow	Irregular	Flat	Gram-positive	Rod
Iso 1 O <sub>2</sub>	White	Circular	Raised	Gram-positive	Cocci

**Table 3.** Colony and bacterial characteristics of isolates from anaerobic conditions.

Isolate	Colony Characteristics			Bacterial Characteristics	
	Color	Form	Elevation	Gram Stain	Shape
Sol'n An 1	Creamy	Circular	Flat	Gram-negative	Cocci
Sol'n An 2	White	Circular	Flat	Gram-negative Gram-positive	Rod Cocci
Sol'n An 3	Yellow	Circular	Flat	Gram-positive	Rod
WW An 1	White	Circular	Flat	Gram-negative	Rod
WW An 2	Creamy	Circular	Flat	Gram-negative Gram-positive	Rod Cocci
WW An 3-1	Creamy	Circular	Flat	Gram-negative	Rod
WW An 3-2	Yellow	Circular	Raised	Gram-negative	Rod
WW An 4	Yellow	Circular	Flat	Gram-negative	Rod
Sludge An 1	Creamy	Irregular	Flat	Gram-negative	Cocci
Sludge An 2	Creamy	Irregular	Flat	Gram-negative Gram-positive	Rod Rod
Iso 2 An	Faint Orange	Circular	Flat	Gram-negative	Cocci



**Figure 5.** Chemical Oxygen Demand reduction performance of Anoxic-oxic system.



**Figure 6.** Chemical Oxygen Demand reduction performance of the Anoxic-oxic-oxic system.



**DISCUSSION**

**Plastic Recycling Wastewater Characteristics**

Table 1 shows the wastewater characteristics of the plastic recycling wastewater from local plastic recycling facilities in the Philippines as compared to the Class C general effluent guidelines set by the DENR. The plastic recycling industry falls under the Philippine Standard Industrial Classification Code (PSIC Code) 38210. Based on DENR Administrative Order 2016-08, the significant parameters that should be monitored for the effluent of industries under PSIC Code 38210 are pH, temperature, color, COD, total coliform, ammonia, nitrate, phosphate, and TSS. The BOD in the wastewater (651 mg L<sup>-1</sup>) was significantly higher than the effluent standard set by the Department of Environment and Natural Resources (DENR) (50 mg L<sup>-1</sup>). This result indicates that the wastewater has a high level of organic matter, which can deplete oxygen levels in water bodies. The COD of the wastewater (5682 mg L<sup>-1</sup>) was also found to be significantly higher than the DENR effluent standard (100 mg L<sup>-1</sup>). This high COD level suggests a substantial presence of organic pollutants in the wastewater, which can have adverse effects on the receiving water bodies (Tchobanoglous et al. 2003). Also, nitrate concentration in the wastewater (337 mg L<sup>-1</sup>) was above the DENR effluent standard (14 mg mg L<sup>-1</sup>). High levels of nitrates can pose a risk to aquatic life and can also contaminate groundwater (Tay et al.

2003). The total suspended solids (TSS) of the wastewater (338 mg L<sup>-1</sup>) also exceeded the DENR effluent standard (100 mg L<sup>-1</sup>). High levels of TSS can reduce the clarity of water, making it unsuitable for various purposes, including industrial and agricultural uses (Tchobanoglous et al. 2003). The phosphate concentration (3.16 mg L<sup>-1</sup>) was observed to be barely below the DENR effluent standard (4 mg L<sup>-1</sup>). High levels of phosphate can lead to eutrophication, which can cause algae blooms and oxygen depletion in water bodies (Tay et al. 2003). The fecal coliform, color, pH, and temperature of the plastic recycling wastewater were well below the effluent standards. However, it has higher concentrations of nitrates (337 mg L<sup>-1</sup>), BOD (651 mg L<sup>-1</sup>), TSS (338 mg L<sup>-1</sup>), and phosphates (3.16 mg L<sup>-1</sup>) than the general effluent standards. This data proves that it is unsafe to discharge wastewater from these industries without proper treatment.

The plastic recycling wastewater from the local industry had higher concentrations of COD than the wastewater from all the characterized wastewaters (Table 4). Also, this wastewater had higher BOD than the wastewater from the studies of Kolbl (2016) and Setiawan et al. (2021). The plastic recycling wastewater also had higher concentrations of nitrates than the study of Altieri et al. (2021). However, it also contains lower concentrations of phosphates than the wastewater from the study of Kolbl (2016) and had a lower pH than all the wastewater (Table 4).

**Table 4.** Wastewater characteristics of plastic recycling industry wastewater from different sources.

Source	Wastewater	COD (mg L <sup>-1</sup> )	TSS (mg L <sup>-1</sup> )	BOD <sub>5</sub> (mg L <sup>-1</sup> )	TN (mg L <sup>-1</sup> )	TP (mg L <sup>-1</sup> )	NO <sub>2</sub> -N (mg L <sup>-1</sup> )	NO <sub>3</sub> -N (mg L <sup>-1</sup> )	NH <sub>3</sub> -N (mg L <sup>-1</sup> )	pH
Altieri et al. (2021)	Plastic solid waste washing wastewater	1371.5	-	398	45.35	8.4	0.1	0.55	22.75	7.6
Setiawan et al. (2021)	Polyester synthetic fiber industry wastewater	579	269	264	-	-	-	-	5.4	-
Santos et al. (2005)	Effluents from washing steps of polyethylene terephthalate (PET) and polyolefins cleaning process	677.5	702.5	-	-	-	-	-	-	9.45
Ramirez-Camperos et al. (2004)	Bottle washings wastewater from soft drinks industry	64.9	66	-	-	-	-	-	-	11.5
Santhmayor et al. (2020)	Wastewater from waste plastic recycling machines	354	400	-	-	-	-	-	-	7.9
Kolbl (2016)	Plastic processing plant wastewater	457	294.3	112.3	-	933.3	248.3	940	456.3	7.6

### Isolated Bacteria in Activated Sludge

There are seven unique bacteria colonies identified in aerobic plates. There are four gram-negative bacterial isolates and three gram-positive bacterial isolates from the aerobic conditions. The color of the colonies of these isolates ranges from white to yellow. Most isolates found in aerobic conditions are rod-shaped with one isolate having a cocci shape. The bacteria colonies isolated from aerobic plates are potential nitrifying bacteria as well as biological phosphorous removal bacteria (Tay et al. 2003). Meanwhile, there are 11 unique bacterial colonies identified in anaerobic plates. Seven are found to be gram-negative while the other four are gram-positive. The color of the colony of these isolates ranges from white to yellow as well. Five isolates have a cocci shape, while the rest are rod-shaped. These bacterial colonies in anaerobic plates can serve as denitrifiers in the anoxic or anaerobic chambers of wastewater treatment (Tay et al. 2003). The presence of both aerobic and anaerobic bacterial colonies gives insight into an effective biological treatment of wastewater.

Out of the 18 isolates, 11 are gram-negative and 7 are gram-positive. The majority of the isolates being gram-negative is an indication that the bacterial community found in the activated sludge is composed of bacteria that are commonly found in wastewater treatment facilities. *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Acidobacteria* are generally found in wastewater treatment systems and are gram-negative (Hu et al. 2012). Meanwhile, the gram-positive isolates may be due to fecal coliforms such as *Enterococcus faecalis* (He et al. 2021).

### Chemical Oxygen Demand Reduction Performance of the AO and AO<sub>2</sub> Systems

The laboratory-scale reactors were used to treat synthetic wastewater. Chemical oxygen demand was measured every day for 10 days using the DNS method. The AO system had a minimum COD reduction rate of 56.20 mg L<sup>-1</sup> h<sup>-1</sup>, a maximum of 60.24 mg L<sup>-1</sup> h<sup>-1</sup>, and a mean of 59.88 mg L<sup>-1</sup> h<sup>-1</sup>. The system also had a minimum removal of 92.10% COD, a maximum of 99.37%, and a mean of 98.13%. The AO system showed performance stability during the 10-day experiment with a decrease in both the COD reduction rate and percent COD removal on the 8<sup>th</sup> day. However, it stabilized again to a comparable COD reduction rate and percent COD removal on the 9<sup>th</sup> and 10<sup>th</sup> day.

Meanwhile, the AO<sub>2</sub> system had a minimum reduction rate of 24.55 mg l<sup>-1</sup> h<sup>-1</sup> COD, a maximum of 39.35 mg L<sup>-1</sup> h<sup>-1</sup>, and a mean of 35.55 mg L<sup>-1</sup> h<sup>-1</sup>. The system also had a minimum removal of 58.92% COD, a maximum of 94.44%, and a mean of 85.33%. The AO<sub>2</sub> system showed performance stability until the 5<sup>th</sup> day. However, it experienced an erratic performance

from the 6<sup>th</sup> day to the 10<sup>th</sup> day, with a characteristic decrease and then an increase in both the COD reduction rate and percent COD reduction. The erratic performance may be due to the change in pH in the reactor from the 6<sup>th</sup> day until the 10<sup>th</sup> day of the experiment.

Overall, the AO system performed better than the AO<sub>2</sub> system in removing COD from the wastewater. The percent COD removal of the AO system as compared to other systems has a comparable percent COD removal. In Table 5, a comparable percent COD removal was observed in the treatment of raw washing wastewater using a sequencing batch biofilter granular reactor with a removal of 95.24% COD and in the treatment of synthetic wastewater using an intermittently aerated and decanted single-reactor process with a removal of 95% COD (Yoo et al. 1999; Altieri et al. 2021). Meanwhile, the AO<sub>2</sub> system has comparable percent COD removal performance with the coagulation-flocculation process, with 82.58% COD removal (Setiawan et al. 2021). Both the AO system and the AO<sub>2</sub> system have significant COD reduction rates compared to other systems (Balku 2007; Munz et al. 2007; Liu et al. 2013; Kim et al. 2019; Nikmanesh et al. 2018; Altieri et al. 2021).

The above results showed the potential of biological treatment for treating plastic recycling wastewater. To further enhance the removal efficiencies of biological treatment systems, it is recommended to examine and optimize the different operating parameters of the systems, such as the pH, the HRT, the dissolved oxygen content, and the influent flowrate of the system. It is also important to upscale the system and evaluate and validate the laboratory results as compared to pilot-scale treatment efficiencies.

### FUNDING

This research is funded by the Department of Science and Technology (DOST) - Philippine Council for Industry, Energy and Emerging Technology Research and Development (PCIEERD).

### ETHICAL CONSIDERATIONS

No human or animals were involved in the conduct of the study.

### DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests for any authors.



**Table 5.** Chemical oxygen demand (COD) and percent COD removal of different systems from various sources.

Wastewater	System	COD Reduction Rate (mg L <sup>-1</sup> h <sup>-1</sup> )	Percent COD Removal	Source
Raw washing wastewater	Sequencing batch biofilter granular reactor (SBBGR)	27.00	95.24	Altieri et al. (2021)
Tannery wastewater	Activated sludge process that was enhanced by coagulation and reverse osmosis	123.97	67.00	De Gisi et al. (2009)
Septic tank effluent	Circulating fluidized bed bioreactor	18.23	78.48	Liu et al. (2013)
Polyester synthetic fiber industry wastewater	Coagulation-flocculation	209.42	82.58	Setiawan et al. (2021)
Tannery wastewater	Powdered activated carbon and membrane bioreactors (MBRPAC)	42.92	79.46	Munz et al (2007)
Synthetic wastewater	Intermittently aerated and decanted single-reactor process	12.44	95.00	Yoo et al. (1999)
Synthetic wastewater	Conventional activated sludge system	32.46	70.11	Balku (2007)
Municipal wastewater	Circulating fluidized bed bioreactor	126.29	92.08	Patel et al. (2006)
Actual WWTP influent	Aerobic-anoxic process	10.54	89.53	Kim et al. (2019)
	Activated sludge process	8.86	75.19	
Municipal wastewater	UASB (Upflow anaerobic sludge blanket) - Activated sludge system	61.42	89.67	Von Sperling et al. (2001)
Municipal wastewater	Extended aeration activated sludge system	2.21	61.40	Nikmanesh et al. (2018)
Synthetic plastic recycling wastewater	Anoxic-oxic system	59.88	98.13	This study
Synthetic plastic recycling wastewater	Anoxic-oxic-oxic system	35.55	85.33	

## ACKNOWLEDGMENTS

This study is part of the DOST PCIEERD funded project entitled “ENVITECS: NanoSiliCage (NSC): Nanocaged silicate composite for treatment of wastewater from Valenzuela City plastic industries” in collaboration with Material Science and Engineering Program – UP Diliman and Department of Engineering Science – UP Los Baños. We also want to express our gratitude to the reviewers for their insightful comments and suggestions, which helped us improve the quality of our research paper.

## REFERENCES

- Adney B and Bake J. 2008. Measurement of Cellulase Activities Laboratory Analytical Procedure (LAP). U.S. Department of Energy and Office of Energy Efficiency and Renewable Energy. 8pp. <https://www.nrel.gov/docs/gen/fy08/42628.pdf>. Accessed on 22 May 2023.
- Altieri VG, De Sanctis M, Sgherza D, Pentassuglia S, Barca E and Di Iaconi C. 2021. Treating and reusing wastewater generated by the washing operations in the non-hazardous plastic solid waste recycling process: Advanced method vs. Conventional method. *Journal of Environmental Management*, 284: 112011. <https://doi.org/10.1016/j.jenvman.2021.112011>
- Alzate-Gaviria LM, Pérez-Hernández A, Nevárez-Moorillón VG, Rinderknecht-Seijas N and Poggi-Varaldo HM. 2003. Comparison of two anaerobic coupled systems for biomethanization of the organic fraction of municipal solid wastes. *Interciencia*, 28(8): 436-490.
- Aryal S. 2022. Plate Count Agar (PCA)- Composition, Principle, Preparation, Results, Uses. <https://microbenotes.com/plate-count-agar-pca/>. Accessed on 29 May 2023.
- Balku S. 2007. Comparison between alternating aerobic-anoxic and conventional activated sludge systems. *Water Research*, 41(10): 2220–2228. <https://doi.org/10.1016/j.watres.2007.01.046>
- Curtin K, Duerre S, Fitzpatrick B and Meyer P. 2011. Biological nutrient removal manual. <https://www.pca.state.mn.us/sites/default/files/wq-wwtp8-21.pdf>. Accessed on 29 May 2023.
- De Gisi S, Galasso M and De Feo G. 2009. Treatment of tannery wastewater through the combination of a conventional activated sludge process and reverse osmosis with a plane membrane. *Desalination*, 249(1): 337–342. <https://doi.org/10.1016/j.desal.2009.03.014>
- Gunarathna GPN, Bandara NJGJ and Liyanage S. 2010. Analysis of Issues and Constraints Associated with Plastic Recycling Industry in Sri Lanka. Department of Forestry and

- Environmental Science University of Sri Jayawardanepura, Nugegoda, Sri Lanka, 11pp.
- He J, Zheng Z and Lo IMC. 2021. Different responses of gram-negative and gram-positive bacteria to photocatalytic disinfection using solar-light-driven magnetic TiO<sub>2</sub>-based material, and disinfection of real sewage. *Water Research*, 207: 117816. <https://doi.org/10.1016/j.watres.2021.117816>
- Hopewell J, Dvorak R and Kosior E. 2009. Plastics recycling: Challenges and opportunities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1526): 2115–2126. <https://doi.org/10.1098/rstb.2008.0311>
- Hu M, Wang X, Wen X and Xia Y. 2012. Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. *Bioresource Technology*, 117: 72–79. <https://doi.org/10.1016/j.biortech.2012.04.061>
- Kolbl S. 2016. Biological wastewater treatment of different organic loading from recycling and fabrication plant of PET, HDPE and LDPE plastic products. *Acta Hydrotechnica*, 29(51): 125-143.
- Kim IT, Lee YE, Yoo YS, Jeong W, Yoon YH, Shin DC and Jeong Y. 2019. Development of a combined aerobic–anoxic and methane oxidation bioreactor system using mixed methanotrophs and biogas for wastewater denitrification. *Water*, 11(7): 1377. <https://doi.org/10.3390/w11071377>
- Liu G, Xu X, Zhu L, Xing and Chen J. 2013. Biological nutrient removal in a continuous anaerobic–aerobic–anoxic process treating synthetic domestic wastewater. *Chemical Engineering Journal*, 225: 223–229. <https://doi.org/10.1016/j.cej.2013.01.098>
- Munz G, Gori R, Mori G and Lubello C. 2007. Powdered activated carbon and membrane bioreactors (MBRPAC) for tannery wastewater treatment: long term effect on biological and filtration process performances. *Desalination*, 207(1–3): 349–360. <https://doi.org/10.1016/j.desal.2006.08.010>
- Nasr Esfahani K, Zandi MD, Travieso-Rodriguez JA, Graells M and Pérez-Moya M. 2021. Manufacturing and Application of 3D Printed Photo Fenton Reactors for Wastewater Treatment. *International Journal of Environmental Research and Public Health*, 18(9): 4885. <https://doi.org/10.3390/ijerph18094885>
- Nikmanesh M, Eslami H, Momtaz SM, Biabani R, Mohammadi A, Shiravand B and Mahmoudabadi TZ. 2018. Performance evaluation of the extended aeration activated sludge system in the removal of physicochemical and microbial parameters of municipal wastewater: a case study of Nowshahr wastewater treatment plant. *Journal of Environmental Health Sustainable Development*, 3(2): 509-17
- Ogodo AC, Agwaranze DI, Daji M and Aso RE. 2022. Chapter 13 - Microbial techniques and methods: basic techniques and microscopy. In: Egbuna C, Patrick-Iwuanyanwu KC, Shas MA and Ifemeje JC (eds). *Analytical Techniques in Biosciences: From Basics to Applications*. Elsevier Science, Amsterdam, The Netherlands. pp 201-220. <https://doi.org/10.1016/B978-0-12-822654-4.00003-8>
- Patel A, Zhu J and Nakhla G. 2006. Simultaneous carbon, nitrogen and phosphorous removal from municipal wastewater in a circulating fluidized bed bioreactor. *Chemosphere*, 65(7): 1103–1112. <https://doi.org/10.1016/j.chemosphere.2006.04.047>
- Ramirez Camperos E, Mijaylova Nacheva P and Diaz Tapia E. 2004. Treatment techniques for the recycling of bottle washing water in the soft drinks industry. *Water Science and Technology*, 50(2): 107–112. <https://doi.org/10.2166/wst.2004.0101>
- Santhmayor KD, Shiri ND, Asiya I, Krafft MS and Thurm W. 2020. Development of water filtration unit for wastewater generated from waste plastics recycling machines. *AIP Conference Proceedings*. 15pp.
- Santos ASF, Teixeira BAN, Agnelli JAM and Manrich S. 2005. Characterization of effluents through a typical plastic recycling process: An evaluation of cleaning performance and environmental pollution. *Resources, Conservation and Recycling*, 45(2): 159–171. <https://doi.org/10.1016/j.resconrec.2005.01.011>
- Setiawan Y, Taufik Rizaludin A, Nur Aini M and Saepuloh S. 2021. Chemical Treatment in Industrial Wastewater of Polyester Synthetic Fiber Made from Recycled Polyethylene Terephthalate Bottles: Minimize Environmental Impacts. *Iranian Journal of Energy and Environment*, 12(3): 192–197. <https://doi.org/10.5829/IJEE.2021.12.03.02>
- Tay JH, Chui PC and Li H. 2003. Influence of COD:N:P Ratio on Nitrogen and Phosphorus Removal in Fixed-Bed Filter. *Journal of Environmental Engineering*, 129(4): 285–290. [https://doi.org/10.1061/\(ASCE\)0733-9372\(2003\)129:4\(285\)](https://doi.org/10.1061/(ASCE)0733-9372(2003)129:4(285))
- Tchobanoglus G, Burton F and Stensel HD. 2003. *Wastewater engineering: treatment and reuse*. American Water Works Association. Journal, 95(5): 201.
- von Sperling M, Freire VH and de Lemos Chernicharo CA. 2001. Performance evaluation of a UASB - activated sludge system treating municipal wastewater. *Water Science and Technology*, 43(11): 323–328. <https://doi.org/10.2166/wst.2001.0698>
- Yoo H, Ahn KH, Lee HJ, Lee KH, Kwak YJ and Song KG. 1999. Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in an intermittently-aerated reactor. *Water Research*, 33(1): 145–154. [https://doi.org/10.1016/S0043-1354\(98\)00159-6](https://doi.org/10.1016/S0043-1354(98)00159-6)

**ROLE OF AUTHORS:** JUT - data collection, data gathering, data analysis, manuscript writing, and editing; JSV – conceptualization, supervision, manuscript writing, and editing.