



Effects of Blended Enzymes, Organic Catalysts, and Probiotics on the Water Quality of Pasig River, Philippines

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ABSTRACT

The study was conducted to evaluate the effectiveness of the blend of enzymes, organic catalysts, and probiotics on the water quality of Pasig River and to determine its sustainability to aquatic life. The effects of varying the dosage of the mixture were also identified. The data observed were compared to the standard for fishery water set by the Department of Environment and Natural Resources (DENR). On-site application results were analyzed using paired t-test. All parameters were normally distributed. Temperature, phosphate, and BOD measures were significantly changed at ($p < 0.05$). Results showed mixture effectiveness on temperature, pH, Chloride, Nitrate, TSS, Oil and Grease, and Surfactant as they passed the DAO 2016-08 Class C Standard. BOD, Phosphate, TSS, and Ammonia concentrations were reduced in Ayala samples. DO, Nitrate and Fecal Coliforms concentrations were reduced in Nagtahan samples. Varying the amount of mixture application yields no significant effect on the water quality. Fishes and aquatic life can still not thrive on this kind of water.

INTRODUCTION

Pasig River was previously one of the most economically important rivers in the Philippines. The drastic development of urban districts around the world has caused changes in the environment (Guzman et al, 2020). Department of Environment and Natural Resources (DENR) and Ecosystem Research and Development Bureau (ERDB) shows that fishes such as tilapia (*Oreochromis niloticus*), Manila Sea catfish (*Arius manillensis*), bighead carp (*Hypophthalmichthys nobilis*), Indo-Pacific tarpon (*Megalops cyprinoides*), and other fishes were found to be living in the river as well as the aquatic plants and other vegetation. Due to development, trade and commerce had significantly grown leading to over migration of inhabitants, this created discharges of domestic and industrial waste which are impacted by various contaminants and toxins such as suspended solids, biodegradable organics, pathogens, nutrients, refractory organics, priority pollutants, heavy metals, and dissolved inorganics (Tawfik et al, 2022). Now, the Pasig River is declared a “biologically dead” river. Physical and

chemical methods have not been adequate to treat this type of ecosystem and the result of long-occurring pollutants in water would include immunosuppression, reduced metabolism, and damage to gills and epithelia, and, ultimately, these can cause fish mortality (Iftikhar et al, 2022). For the flora and fauna of the Pasig River to thrive and survive, the quality of water should be considered. Today, bioremediation had emerged as a sustainable and recognized innovative technology to handle contaminants (De Lima et.al, 2018).

Bioremediation is a process to detoxify contaminants in water, soil, or other environments through the application of microorganisms, and plants, blending of enzymes, organic catalysts, and probiotics (Sellami et al, 2021; Bwapwa, 2022). The mixture of a combination of enzymes, organic catalysts, and probiotics is a beige-colored powder with enzymes such as amylase, protease, cellulose, pectinase, phytase, and probiotic content of 90,000,000,000 colony-forming units per kilogram of microencapsulated organism *Bifidobacterium longum*, *Bifidobacterium thermophilum*, *Bacillus*

subtilis, and *Lactobacillus acidophilus*. There was already a successful treatment done on contaminated water and soil sites of aquatic ponds and lagoons of swine and poultry farms using this mixture. It helps in the improvement of water quality by breaking down organic matter and restoring aerobic conditions to bodies of water for aquatic life revival and eliminating organic matter and inorganic pollutants and bringing life back to the bodies of water (Sanchez et al, 2016).

The benefits of this combination, between enzymes, organic catalysts, and probiotics, are to improve water quality parameters including COD, BOD, TDS, TSS, and ammonium levels. It also eliminates suspended solids to clear up water for photosynthesis. Ammonia and hydrogen sulfide are known toxic gas produced during the growth cycle, it is also controlled by the combination of this mixture to eliminate harmful effects on aquatic life and improve the air quality of the place (Ibrahim, 2015). In addition, the oxidation of ponds to anaerobic processes and the prevention of the formation of algae is also a benefit of this combination. It also breaks down organic compounds such as ammonia to protein increasing nutrients and purifying the water faster and allowing the compost for a good quality fertilizer (Abdel-Raouf et al, 2012).

The study was conducted to evaluate the effectiveness of the blend of enzymes, organic catalysts, and probiotics on the water quality of Pasig River and determine the river's ability to sustain aquatic life. Specifically, the study aimed to determine the changes in water in Pasig River after treatment based on parameters: temperature, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chloride, nitrate as $\text{NO}_3\text{-N}$, phosphate, total suspended solids (TSS), fecal coliforms, ammonia $\text{NH}_3\text{-N}$, oil and grease, surfactants, conductivity, turbidity, total dissolved solids (TDS). This study also identifies the effects of varying the dosage and frequency of application treated by the blends of enzymes, organic catalysts, and probiotics in the improvement of water quality. Lastly, the data from this study were compared with the data obtained with the standard for fishery water

set by the Department of Environment and Natural Resources (DENR).

MATERIALS AND METHODS

The on-site pilot application started with baseline water quality monitoring. Then a concentrated blend of enzymes, organic catalyst, and probiotics (Oxydol® by Leads Agri Animal Health, Agranco Corp.) was prepared by mixing 1 kilogram of the powdered combination in 5 liters of water and then further diluted to 200 liters of water and was applied gradually, on a zigzag manner within the 2 km stretch of main Pasig River along Nagtahan Bridge by allowing it to flow from a hose intermediate bulk container tank on a boat. The solution was mixed until it homogenized and forms a brownish mucoid fluid. Water quality monitoring and sampling were implemented. First-week of application was done in 2020, June 25th, 27th, and 29th with a total of 15 kg of blend mixture; enzyme, catalyst, and probiotics. The second application was done on July 2nd and 3rd with 12 kg of the mixture while the third-week application was performed on the 9th, 11th, and 13th day of July with 9 kg of the mixture while 6 kg of the mixture was given on the fourth week, on July 19th and 20th. The dosage was decreased by 3 kg every week as part of the product protocol. A total of 42 kg of a blend of enzymes, catalysts, and probiotics were dosed for the whole month of implementation. Water quality monitoring was performed on 2020, June 25th, July 9th, 20th, and 27th.

RESULTS AND DISCUSSION

The on-site application of the blend of enzymes, organic catalysts, and probiotics along Pasig River started on June 25th wherein weather in all stations was sunny with a downward flow direction and wind condition was slightly breezy. Weather on July 9th was also sunny with a downward flow of water and slight breezy wind condition. The third collection date, which was July 20th, had rainy weather with a downward flow of water and windy wind condition. The last collection date was July 27th, wherein weather condition was cloudy with downward water flow and slight breezy wind condition.

Table 1. List of Parameters that Assessed Water Quality upon On-site Application of Blends of Enzymes, Organic Catalysts and Probiotics to Whether Passed or Failed based on the DAO 2016-08 Class C Standard

Parameters	Unit	Class C	Ayala Bridge	Mabini Platform	Nagtahan Bridge
		Water Quality Standard			
Temperature	°C	25-31	28.34±1.21*	28.33±0.84*	28.28±1.00*
pH	(range)	6.5-9.0	6.89±0.50*	7.09±0.65*	7.15±0.18*
Dissolved Oxygen	mg/L	5	1.88±0.04	2.28±0.04	2.03±0.03
Biochemical Oxygen Demand	mg/L	7	29.75±1.15	31.50±1.20	36.50±0.95
Chloride	mg/L	350	231.7±12.5*	107.50±14.5*	104.0±10.5*
Nitrate as NO ₃ -N	mg/l	7	1.12±0.02*	1.24±0.02*	1.15±0.02*
Phosphate	mg/L	0.5	1.35±0.03	1.39±0.04	1.39±0.03
Total Suspended Solids	mg/L	80	17.0±1.25*	20.25±1.50*	20.75±1.30*
Fecal Coliforms	MPN/100ml	200	2010000.0	1220000.0	1029750.00
Ammonia as NH ₃ -N	mg/L	0.05	2.24±0.02	2.27±0.03	2.32±0.03
Oil and Grease	mg/L	2	1.32±0.01*	1.85±0.02*	1.33±0.02*
Surfactants	mg/L	1.5	0.66±0.01*	0.49±0.01*	0.91±0.02*

(Sampling Station= Ayala bridge, Mabini Platform, Nagtahan bridge)

*- Passed the Class C Standard of DAO 2016-08

Table 2. Percentage of concentration changes after the application of the treatment

Parameters	Ayala Bridge	Mabini Bridge	Nagtahan Bridge
Ammonia	-5.79±0.52%*	+19.46±0.89%	+8.12±0.45%
Biochemical Oxygen Demand	-46.34±1.11%*	-27.03±1.55%	-30.43±1.46%
Dissolved Oxygen	-5.00±1.00%	-28.57±2.24%	+35.71±2.59%*
Nitrate	-15.87±1.45%	-4.69±0.04%	-22.56±0.88%*
Phosphate	-33.72±1.29%*	-24.53±1.74%	-32.74±2.00%
Total Suspended Solids	-54.84±4.55%*	-8.70±0.98%	-50.00±2.87%
Fecal Coliforms	-85.88±5.64%	-83.53±4.32%	-96.41±7.12%

*-highest percent change

The probiotics alter the normal bacterial flora of the wastewater instead of the virus, pathogenic and antibiotic-resistant bacteria due to the antiviral and antibacterial effect of probiotics. Probiotic bacteria directly uptake or decompose the organic matter or toxic material in the water thus, improving water quality (Padmavathi et al, 2012). Enzymes present in the treatment may speed up the process of decomposition as well as break down organic compounds.

Temperature

The temperature of the water varies along the length of the river with latitude and elevation, and between small sections only a few meters apart depending on local conditions (Yang & Peterson et al, 2022). It was shown that the temperature of water fluctuated between day and night and over longer periods. It ranged from 25.02 to 30.70°C across sampling stations which was within the Class C Standards as prescribed in DAO 2016-08.

Temperature changes indicated that weather condition changes during the onsite pilot application period. Based on statistics, there was a significant decrease from the first week of application to the fourth week ($P < 0.05$). The temperature of the water is inversely related to Dissolved Oxygen (DO) concentration, every change in temperature affects the river's ability to self-purify by lowering the amount of oxygen that can be dissolved and utilized for biodegradation (Rajesh & Rehana *et al.*, 2022).

pH

The pH of river water is a measure of hydrogen ion concentration that indicates the acidic or basic water condition. The optimum pH for river water is around 7.4. Water's acidity can be increased by acid rain. Extremes in pH can make a river inhospitable to life and speed the leaching of heavy metals harmful to fish and insects (Wang *et al.*, 2022). As observed, the pH in all sampling stations fluctuates since the environmental and weather conditions were changing during the entire application. It ranges from 6.30 to 7.69. Dramatic changes in pH may be due to precipitation especially acid rain, wastewater or mining discharges, and carbon dioxide concentrations in the study by Hickin (1995). Generally, the pH recorded in all sampling stations passed the Class C Standards for pH (6.5-9) except at Ayala Bridge station during July 20th and July 27th sampling in which the measured pH was 6.38 and 6.30, respectively.

Dissolved Oxygen (DO)

The amount of dissolved oxygen in water depends on the water temperature, salinity, pressure, water flow, seasonal change, altitude, total suspended solids, total dissolved solids, nutrients, water saturation, and photosynthesis (Wilson, 2010). The decrease in dissolved oxygen may be due to the increase in temperature as the solubility of oxygen decreases as temperature increases, the same result was also seen in a study by Wetzel (2001). On the other hand, the increase in dissolved oxygen level may be caused by weather conditions as during the wet season, dissolved oxygen increases because rainwater interacts with the atmospheric oxygen same explanation was given in the study on Hard & Soft Water (2016). The low level of dissolved oxygen in water is a sign of contamination and is an important factor in determining water quality, pollution control, and

treatment process (Bozorg-Haddad *et al.*, 2021). In addition, oxygen concentrations were much affected by the volume and flow speed of the water, the greater the speed, the higher the dissolved oxygen level. Areas with slower-flowing water will have fewer aerobic organisms because of the difficulty to survive in such difficult conditions. The faster flow makes it more difficult for organisms to control their interaction with their environment (Felder *et al.*, 2019). Moreover, the increase in dissolved oxygen can also be the result of the blend of enzymes, organic catalyst, and probiotics application as the dissolved oxygen had increased in the Nagtahan Bridge sampling station where the solution was directly applied. The increase in dissolved oxygen was due to the proliferation of probiotics which increase the rate of decomposition of organic matter in wastewater (Sanchez *et al.*, 2016). Results implied that dissolved oxygen concentrations in all sampling stations during the entire onsite pilot application were low and failed to reach the guideline for Class C waters which is 5 mg/L.

Biochemical Oxygen Demand (BOD)

As observed, the (BOD) in all sampling stations reduced during the entire onsite pilot application by 46.34%, 27.03%, and 30.43% (Table 2) for Ayala Bridge sampling station, Mabini Platform sampling station, and Nagtahan sampling station, respectively; however, it failed to reach the Ambient Water Quality Guideline for Class C level which is 7 mg/L. A decrease in BOD means an increase in dissolved oxygen which also manifested in the results discussed earlier. Based on statistics, there was a significant decrease in BOD concentration from the first week of application to the fourth week at a 5% level of significance. High BOD concentrations reduce oxygen availability, degrade aquatic habitats and biodiversity, and impair water use (Vigiak *et al.*, 2019).

Chloride

Results showed that a continuous reduction of chloride was observed in all sampling stations as the onsite pilot application progresses; 88.85% reduction in Ayala Bridge, 53.90% in Mabini Platform, and 40.32% in Nagtahan Bridge, and these chloride concentrations recorded passed the water quality guidelines as stated in DAO 2016-08 for Class C water which was 350 mg/L. High chloride concentrations can increase the tendency of

water to cause corrosion in distribution systems which is a costly problem in water distribution (Stets et al., 2018).

Nitrate as $\text{NO}_3\text{-N}$

The nitrate levels in all sampling stations during the entire monitoring period were low ranging from 1.02 to 1.33 mg/L and it passed the water quality standard for Class C which was 7 mg/L. A decreasing trend in the concentration of nitrates was observed throughout the monitoring period in all stations, but it eventually increased towards the end of the monitoring period. A decrease in nitrate concentration can be due to the degradation of contaminants by the application of a blend of enzymes, organic catalysts, and probiotics. Riverine $\text{NO}_3\text{-}$ concentrations are attributed to several potential pollution sources, such as atmospheric deposition nitrate (AD), nitrogen fertilizer (NF), soil nitrogen (SN), municipal sewage (MS), and industrial wastewater, as well as multiple transformations occurring within the nitrogen cycle (e.g., nitrification and denitrification) (Lu et al., 2015, Jin et al., 2018).

Phosphate as P

The measured phosphate concentration in all sampling stations during the entire monitoring period was high and failed to pass the water quality guidelines for Class C waters which were 0.5 mg/L. Despite that, a decreasing trend was observed manifesting an improvement in water quality status. Phosphate concentration was generally reduced by 33.72%, 24.53%, and 32.74% for Ayala Bridge, Mabini Platform, and Nagtahan Bridge sampling stations, respectively. Based on statistics, there was a significant decrease in phosphate concentration from the first week of application to the fourth week ($P < 0.05$). A phosphate level above 0.02 mg/L can trigger eutrophication and threaten the lives of living organisms (Ahmed and Lo, 2020).

Total Suspended Solids (TSS)

Observed TSS in all sampling stations during the entire monitoring period passed the water quality standards for Class C waters which was 80 mg/L. Moreover, a decreasing trend from the first to the third monitoring dates was observed across all sampling stations. The Ayala Bridge sampling station had the highest percentage reduction among the three sampling stations (table 2). The fractions of Pb, Zn, and Cu are associated with suspended solids (Nasrabadi et al., 2016).

Fecal Coliform

Data showed that fecal coliform count increased across all sampling stations on the second monitoring date and then decreased towards the end of the monitoring period. The fecal coliform count has been reduced as shown in table 2 from the second monitoring date to the end of the monitoring period. The highest reduction in the fecal coliform count was observed in Nagtahan Bridge. The decrease in the fecal coliform count can also be attributed to weather, especially during rainy days when microorganisms are washed away to different locations as the volume and flow of water increase. Meanwhile, the fecal coliform count in all the sampling stations generally failed to comply with the water quality standard for Class C water stated in DAO 2016- 08. Pathogenic bacteria are primarily contained in fecal materials, derived from livestock, animals, and human, into surface water through sewage discharge, agriculture, and stormwater runoff. Numbers of fecal coliform bacteria are widely used as microbiological parameters indicating fecal pollution as a parameter to provide basic information on surface water quality (Hong et al., 2010).

Ammonia

Ammonia levels in all sampling points throughout the entire onsite pilot application were almost equal ranging from 2.06 to 2.64 mg/L. These levels were known to be very toxic among freshwater and marine fishes. However, this range was beyond the Ambient Water Quality Guidelines for Class C level which is 0.05 mg/L. High concentrations of ammonia were due to high concentrations of BOD, temperature, and pH levels (Ding et al., 2021). The chronic and acute aquatic life criteria for ammonia by USEPA (2013) were 1.9 and 17 mg/L at temperature 20°C and pH 7.0 and by MEEC (2019) 1.5 and 12 mg/L.

Oil and Grease

Data implied fluctuations in oil and grease concentration were almost the same for Ayala Bridge and Nagtahan Bridge sampling stations ranging from 1.24 to 1.46 mg/L. On the contrary, the amount of oil and grease measured in the Mabini Platform sampling station notably increased during the second and fourth monitoring dates which failed to meet the 2-mg/L Ambient Water Quality Guideline for Class C waters. Changes in

oil and grease concentration were affected by the change in the weather as well as the flow direction of the water. Oil and grease are not soluble in the water phase. Oily wastewaters contain potentially toxic substances which are inhibitory to plant and animal growth, equally mutagenic and carcinogenic to humans such as petroleum hydrocarbons, phenols, and polyaromatic hydrocarbons (Mantey et al., 2020).

Surfactants

The results showed that the concentrations of surfactants across all sampling stations during the entire monitoring period behaved erratically but were generally low which means that the sampling stations roughly passed the water quality guideline for Class C level stated in DAO 2016-08 which was 1.5 mg/L. As opposed to the rest, the observed concentrations of surfactants in Nagtahan Bridge on the last monitoring date is 1.65 mg/L which was above the said indicator. Surfactants are used mainly as surface-active ingredients in cleaning compounds (detergents, shampoos, and others), and in the processing of textiles, pulp, and paper, recycled paper, paint, and plastics (Gomez et al., 2011).

Conductivity

A sudden change in conductivity in a body of water can infer pollution. An increase in conductivity can be due to the additional chloride, phosphate, and nitrate ions coming from agricultural runoff or a sewage leak or discharge while a decrease in conductivity can be due to an oil spill or additional organic compounds in the water since these elements do not break down into ions. Conductivity varies seasonally along with average temperatures and water flow. A study by Fukuwa et al., (1996) also showed that in some rivers, conductivity can be lower during spring when flow volume is highest as compared during winter due to temperature differences. Moreover, seasonal averages are more dependent on temperature and evaporation in bodies of water with little to no inflow as stated by Fondriest Environmental Inc. (2014). The conductivity of water due to water level fluctuations was often directly connected to water flow. In addition, rainfall during the entire onsite pilot had increased the water volume and level ergo lowering the conductivity, the same study by Patillo (1994) also showed the same results. The slight increase in conductivity observed towards the end

of the onsite pilot application may be caused by evaporation as the water level lessens causing the ions present to become concentrated. Meanwhile, the conductivity in all sampling stations during the entire onsite pilot application ranges from 0.27 to 2.62 mS/cm.

Turbidity

Turbidity across all sampling stations during the entire onsite pilot application varies from 52.20 to 84.88 NTU. The literature stated that clear water is typically considered as an indicator of healthy water while it is likely for some streams to have naturally high levels of suspended solids. Gao, (2006) stated that excessive suspended sediments could be an indicator of a sudden increase in turbidity in a previously clear body of water which can impair water quality for aquatic and human life, impede navigation, and increase flooding risks. Moreover, Langland & Cronin, (2003) stated that this can also infer an increase in stream bank erosion that may have a long-term effect on a body of water. McNally & Mehta, (2004) also stated that water flow also affects turbidity. High flow rates keep particles suspended instead of letting them settle to the bottom; thus, turbidity is always present in rivers and other naturally occurring high-flow environments based on the study of Gray et al., (2000). Wetzel, (2001) also stated that weather also affects turbidity as heavy rainfall escalates stream volume and stream flow which results in the re-suspension of settled sediments and erosion of riverbanks. Rainfall can also directly increase the total suspended solids through runoff. A decrease in turbidity can be attributed to the settling of suspended particles.

Total Dissolved Solids (TDS)

Results showed fluctuations in total dissolved solids observed for the entire onsite pilot application ranging from 0.17 g/L to 1.67 g/L. Total dissolved solids plummeted from the first monitoring date to the next in the Ayala Bridge sampling station. Variations in TDS may be due to temperature changes as temperature directly affects conductivity, and conductivity was directly related to total dissolved solids. TDS concentration is the standard for both drinking water (500 mg/L) and agricultural use (1200 mg/L) (Kim et al., 2022). Natural climatic variations in rainfall, wind, temperature, and snowmelt runoff continuously

cause changes in flow and salinity in the river from year to year (Weltz et al., 2014).

CONCLUSION

Results on the onsite application of a blend of enzymes, organic catalysts, and probiotics showed effectiveness on parameters like temperature, pH, Chloride, Nitrate, TSS, Oil and Grease, and Surfactant as they passed the DAO 2016-08 Class C Standard that BOD, Phosphate, TSS, and Ammonia concentration was reduced greatly on Ayala bridge samples. DO, Nitrate and Fecal Coliforms concentration were reduced greatly in the Nagtahan bridge. Factors such as weather, the flow of water, and wind conditions can affect the effectiveness of the mixture. Varying the amount of mixture application on the On-site application yields no significant effect on the improvement of the water quality of Pasig River. It is recommended that in lab-scale experiments, mixing of water before sampling should be done to avoid the concentration of particles in a certain area. The on-site pilot application must be conducted during the dry season to eliminate several environmental factors that may affect the expected results. Meanwhile, the onsite pilot application must be done in the developed tributaries instead of on the Main Pasig River to eliminate the effect of fast-flowing water on the effectiveness of the product. Effects of varying flow rates, cross-mixing, and varying concentrations of pollutants should also be taken into consideration. The water quality to be analyzed in the laboratory may be limited to Dissolved Oxygen, Total Dissolved Solids, Ammonia, Biochemical Oxygen Demand, and Total Suspended Solids. A cost analysis of the product should be done. This study may be used as representative data regarding the water quality of rivers in Metro Manila.

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