# Biochemical Oxygen Demand ($BOD_5$) Reduction Using Chitin Containing Microbes for River to Reef Protection

Nestor Paul P. Ubalde[1*], Arnil C. Emata[1],
Valentina A. Pallo [2] and Greg M. Cubio[1]
[1]Center for Agriculture and Biosystem Technology
[2]College of Science and Technology Education
University of Science and Technology of Southern Philippines – Cagayan de Oro
Cagayan de Oro City, 9000 Philippines
[*]*nestor@ustp.edu.ph*

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## Abstract

*Chitin, a widely abundant biopolymer from insects and crustaceans, along with effective microorganisms (EM), was evaluated for its potential to reduce wastewater pollutants, particularly biochemical oxygen demand ($BOD_5$). This study attempted to address the gap in cost-effective and environmentally friendly wastewater treatment methods. The objective of this study was to assess the combined effect of chitin and EM on $BOD_5$ reduction and other contaminants. Wastewater samples were treated with chitin and EM mixtures, and $BOD_5$ was measured after a five-day incubation at 20 °C. Results showed a significant reduction in $BOD_5$ from 319 to 11 mg/L, after immediate application of the chitin-containing EM in combination with aeration-supplied microdiffusers and moving bed biofilm reactor (MBBR), with an additional decrease in pollutant levels, indicating improved water quality. This approach demonstrated the potential of using chitin and EM with micro diffusers and MBBR for enhanced wastewater treatment and ecosystem protection, especially in river systems where severe pollution is indicated by $BOD_5$ values exceeding 8 mg/L. The findings provide a foundation for sustainable bioremediation practices. Such technology could be considered sustainable and productive for the agricultural sector and the environment, in general since both chitin and EM are renewable and biologically safe and secured.*

***Keywords**:* biochemical oxygen demand, chitin, effective microorganisms microdiffusers, MBBR

## 1. Introduction

Chitin is the second most abundant organic polymer on Earth, with an annual bio production worldwide estimated to be $10^{11}$ tons, spanning all ecosystems (Sanandiya *et al*., 2020). Chitin is derived from insects like the Black Soldier

Fly and Crustaceans like crabs and shrimps. Likewise, effective microorganisms (i.e., microbes), a term introduced in Japan in the 1990s, are an example of biological (bioremediation) methods used to restore the appropriate ecological balance of the ecosystem and the ability of water to self-purify. The mixtures of bacteria, actinomycetes, yeasts, and fungi have been proven to have a synergistic effect to inhibit harmful bacteria by excluding competition to achieve a dominance of the effective species. Chitin, chitosan, and their composites emerged as promising adsorbents due to their low price, abundance, amino and hydroxyl groups, as well as their potential to remove various toxins from wastewater.

Biochemical oxygen demand (also called biological oxygen demand) is the amount of dissolved oxygen needed (i.e., demanded) by aerobic biological organisms to break down organic material present in a given water sample at certain temperature over a specific time period (Kaiser, 1998). The $BOD_5$ value is most commonly expressed in milligrams of oxygen consumed per liter of sample during five days of incubation at 20 °C (Hach *et al.*, 1997) and is often used as a surrogate for the degree of organic pollution of water (Jouanneau *et al.*, 2014; Armiento, 2016). Most pristine rivers will have a five-day carbonaceous $BOD_5$ below 1 mg/L. Moderately polluted rivers may have a $BOD_5$ value in the range of 2 to 8 mg/L (Li and Liu, 2019). Rivers may be considered severely polluted when $BOD_5$ values exceed 8 mg/L (Grover and Wats, 2013). The BOD value is most commonly expressed in milligrams of oxygen consumed per liter of sample during five days of incubation at 20 °C and is often used as a surrogate of the degree of organic pollution of water (Sawyer *et al.*, 2003). BOD can be used as a gauge of the effectiveness of wastewater treatment plan. As such, $BOD_5$ was the primary consideration in this study because it serves as an indicator of how much oxygen is utilized by a natural water sample (phytoplankton and bacteria) in five days at 20 °C (Penn *et al.*, 2009).

In this study, the application of chitin with EM combination was tested to reduce wastewater pollutants that eventually end up in our river system affecting not only fresh water but also the coastal ecosystem. The main objective of this work was for $BOD_5$ reduction, and the effect of chitin and EM on the reduction of other wastewater contaminants in the wastewater treatment facility of the chicken processing plant as an environment-friendly strategy to comply with the wastewater quality requirements of the Department of Environment and Natural Resources Administrative Order Number 2016-08 (DENR AO No. 2016-08) (DENR, 2016).

## 2. Methodology

A strategy to reduce the $BOD_5$ to meet the standard of wastewater quality under DENR AO No.2016-08 (DENR, 2016) and eliminate odors in the premises, canals, and most importantly in the effluents of a 40,000 head-capacity chicken processing plant was tested for almost four years using chitin-containing microbes in solid and liquid state. A total wastewater effluent of 600 $m^3$ is expected to come out of the facility in a span of 24 to 48 h. Given the organic nature of the products and the processes involved, the wastewater is expected to have high levels of $BOD_5$, suspended solids (TSS), as well as ammonia and phosphates. This wastewater is coursed through the plant's treatment facility where the pollutant loads are reduced.

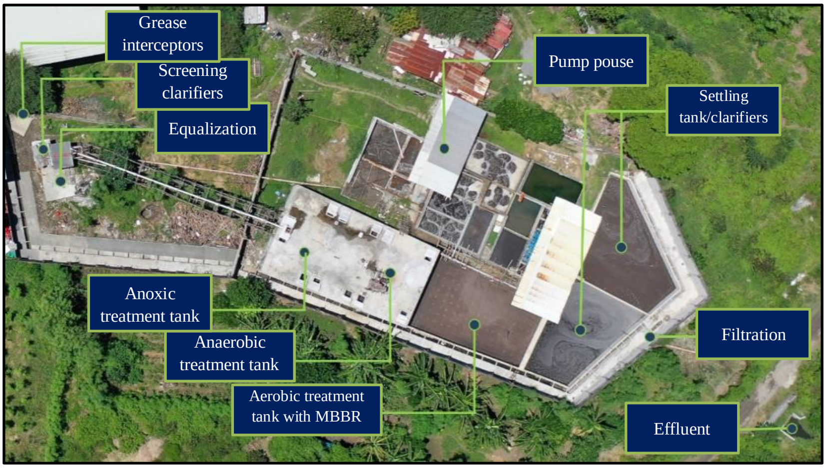


Figure 1. The wastewater treatment facility of the chicken processing plant showing the detailed treatment components

The wastewater generated from the main production facilities of the chicken processing plant goes through primary treatment stages composed of grease interceptors and screens before it is pumped to the wastewater treatment facility (Figure 1).

The wastewater facility where the secondary treatment of the wastewater is employed covers an area of about 500 $m^2$ and is located 5-m away from the main production facility (Figure 2). The secondary treatment is an activated sludge process which consists of a series of sequential batch reactors. These serve to reduce the organic load of the high-strength wastewater and are followed by clarification and filtration before discharge.

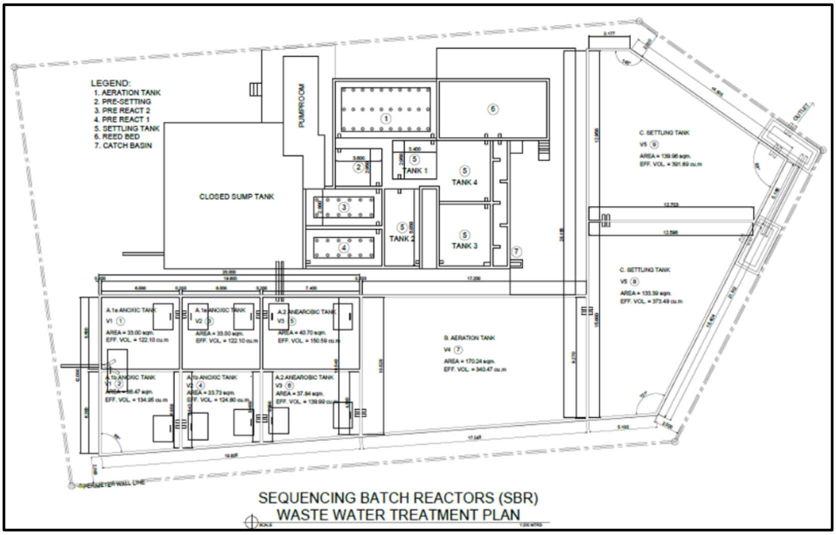


Figure 2. Detailed drawings of wastewater treatment facility of the chicken processing plant indicating the various stages in the sequencing batch reactors

*2.1 Grease Interceptors*

The grease interceptors were installed primarily to reduce the levels of fats, oil, and grease (FOG), which could potentially contribute to the high organic loading of the wastewater. These underwent regular cleaning to ensure that the trapped FOG was removed, scraped off and disposed of regularly.

*2.2 Screening*

Grit screens were also installed to remove feathers, small carcasses, and other solids from the wastewater stream which may result in the clogging of the wastewater pipelines and damage of the pumps and other equipment. The solids were regularly raked off as well and disposed of site with other solid wastes.

*2.3 Equalization*

The filtered wastewater then proceeded by gravity to the sump tank which also served as an equalization tank. This stabilized the variation of the flow and influent characteristics of the wastewater which could lead to shock loading. This was also equipped with submersible pumps that regulated the flow to the sequential batch reactors.

### *2.4 Anoxic Treatment*

The first stage in the series of sequential batch reactors was the anoxic treatment process which was characterized by the absence of molecular or free oxygen within the tank. This process allowed the aerobic microorganisms to break down the nitrogen compounds, particularly nitrates and nitrates, which released the molecular oxygen that allowed the microorganisms to thrive. Nitrogen was given off as nitrogen gas.

### *2.5 Anaerobic Treatment*

The wastewater released from anoxic treatment then underwent further denitrification and breakdown of organic substances through an anaerobic treatment process. This process employed anaerobic microorganisms, such as acidogenic bacteria, acetogenic bacteria, and methanogens, which digested organic compounds in the absence of both free and bound oxygen.

### *2.6 Aerobic Treatment*

Further reduction of organic load in the wastewater occurs through aerobic treatment with chitin containing EM (containing 800,000 cfu/kg) applied at an initial dose of 1 kg per 5 tons of wastewater in parallel with the standard aeration system and with the presence of Moving Bed Biofilm Reactors (MBBR) at a dose of 0.05 kg/ton as suggested by Nourredine and Barjenbruch (2024). The microdiffusers were to provide aeration for the mixing and oxygen supply to the microbes in the degradation of organic matter. MBBR consisting of plastic carriers with large internal surface areas, was an activated sludge aeration system.

### *2.7 Clarifiers/Settling Tank*

As the wastewater existed the aerobic treatment process, it flowed through a setting chamber which allows the fine solids to settle at the bottom. These small solids were still part of the activated sludge and consisted mostly of active bacteria. Part of this activated sludge was returned to the aeration tank to increase the bacterial concentration, helped in propagation, and accelerated the breakdown of organic material while the excess was discarded.

### *2.8 Filtration*

Once the sediments settled, the effluent passed through a packed bed composed of anthracite and activated carbon to further remove contaminants before exiting through the discharge pipe. This process also additionally removed the color and turbidity of the effluent. The effluent was collected in plastic carriers in which the surface area optimizes the contact of water, air, and microorganisms. $BOD_5$ was tested at the DENR-accredited facility.

### *2.9 Analysis*

Wastewater samples were analyzed by a DENR-accredited laboratory in accordance with DENR Administrative Order No. 2016-08, which mandates strict adherence to legal wastewater quality standards in the Philippines. This compliance ensured the proper calibration and maintenance of analytical equipment, enhancing the accuracy and reliability of pollutant measurements. Key parameters analyzed in the study included BOD, nitrates, ammonia, phosphates, and fecal coliform, all of which were critical indicators of wastewater pollution. By following both local regulations and international best practices, the analysis provided robust, scientifically sound data for evaluating environmental impact and informing water quality management.

## 3. Results and Discussion

The use of this integrated strategy lowered the five-day BOD of the wastewater effluent to resulting concentrations of less than 20 ppm in several instances (Figure 3). $BOD_5$ would return to higher values when the strategy is not applied, most especially in the use of the 7.5 and 10 hp blowers and the broadcasting of EM. The liquid application as spray in the perimeter canals eliminated odors from the processing plant. Other parameters showed a great decrease in concentration surpassing the standard set by the DENR under the Class A category. Results revealed that a $BOD_5$ of 13 mg/L was achieved within three months of application and increased to 19 mg/L after six months. The standard limit for this category was 20 mg/L of $BOD_5$. Results also showed that other parameters, such as nitrates, ammonia, phosphate, and fecal coliform, were greatly reduced (up to 99% reduction from starting value).

*3.1 BOD Levels of Waste Effluent*

Figure 3 presents the results of the study that monitored the $BOD_5$ levels in the waste effluent of a chicken processing plant. The baseline BOD concentration of 319 mg/L in September 2019 indicated high organic pollution in the chicken processing plant effluent. This level was well above acceptable environmental standards. The first notable intervention, marked by the yellow arrow in December 2019, involved the introduction of effective microorganisms (EM), a combination of beneficial bacteria, fungi, yeast and actinomycetes aimed at reducing organic matter. After EM treatment, BOD levels dropped significantly to 125 mg/L, demonstrating the initial effectiveness of this biological treatment.


Figure 3. BOD levels of waste effluent of a chicken processing plant

Over the next few months, further reductions were observed, with BOD levels progressively decreasing to 102 mg/L in March 2020 and further down to 50 mg/L by March 2021. This trend indicated that the EM was continuously effective in reducing the organic load. In June 2021, there was a sudden spike in BOD levels to 203 mg/L, marked by a red arrow indicating the removal of aeration. This spike suggested that aeration played a critical role in maintaining lower BOD5 concentrations by promoting aerobic microbial activity.

The researchers then incorporated additional technology such as micro diffusers on July 31, 2021 and MBBR systems. These treatments helped control the spikes, leading to a new low of 11 mg/L in April 2, 2022 after aeration was reintroduced and EM continued to be applied but then each time aeration was removed, BOD levels rose again, as observed in the next two days that spikes to 104 and 120 mg/L in July 2022. The recurring pattern emphasized the importance of maintaining a well-aerated system for optimal treatment.

The combination of aeration, MBBR, and EM provided consistent control of BOD levels. From November 2022 onward, BOD levels remained relatively low, with values around 21 mg/L by September 2023; this marked a reduction from the initial concentration of 319 mg/L.

*3.2 Other Wastewater Pollutants*

Table 1 presents the key indicators of wastewater pollutant reduction through the use of chitin-containing microbes over time, with BOD, nitrates, ammonia, phosphates, fecal coliforms, and overall pollutant reduction measured. Starting on April 4, 2022, BOD was high at 104 mg/L, along with levels of nitrates (96.7 mg/L) and ammonia (96.7 mg/L). However, over time, significant reductions were observed. By September 30, 2023, BOD decreased to 21 mg/L, nitrates to 1.8 mg/L, and ammonia to 1.0 mg/L, showing effective pollutant management.

Phosphate levels followed a similar trend, dropping from 20.4 mg/L to 4.28 mg/L, indicating reduced nutrient pollution. Fecal coliform levels, initially very high at 3,300,000 MPN/100 mL, dropped significantly to 270 MPN/100 mL. Pollutant reduction efficiency was remarkable, consistently above 99.9% for most pollutants, reflecting the effectiveness of the microbial, MBBR, aeration, and chitin treatment in reducing both organic and nutrient pollutants in effluents which contributed to improving water quality in aquatic ecosystems from rivers to reef.

Table 1. Trends of other regulated wastewater parameters required by DENR AO No. 2016-08 and determined during various sampling times during the study

| Date | BOD | Nitrates | Ammonia | Phosphate | Fecal coliform | Pollutant reduction |
|---|---|---|---|---|---|---|
| 4/4/22 | 104 | 96.7 | 96.7 | 20.4 | 3,300,000 | Starting value |
| 7/15/22 | 13 | 0.208 | 1.17 | 20.4 | 300 | 99.99091 |
| 10/26/22 | 19 | 0.625 | 0.019 | 20.1 | 270 | 99.99182 |
| 1/7/23 | 120 | 0.91 | 45.7 | 19.9 | 300 | 99.99091 |
| 2/21/23 | 28 | 0.063 | 0.019 | 17.9 | 1,900 | 99.94242 |
| 4/13/23 | 45 | 3.61 | 1.9 | 2.46 | 2,600 | 99.92121 |
| 9/30/23 | 21 | 1.8 | 1.0 | 4.28 | 270 | 99.99182 |

Figure 4 highlights the trend in wastewater pollutant and BOD reduction over time, showing significant fluctuation. It started at 104 mg/L on 4/4/22 and was gradually reduced, reaching as low as 13 mg/L on 7/15/22. The BOD reduction percentages indicated the effectiveness of the treatment process over time. It showed improvement, with the highest reduction at 88% on 7/15/22, though a significant spike in BOD occurs on 1/7/23, which correlated with a negative reduction (-15%), indicating a possible treatment failure or overload. The system recovered after this, with BOD reduction reaching 80% by 9/30/23.

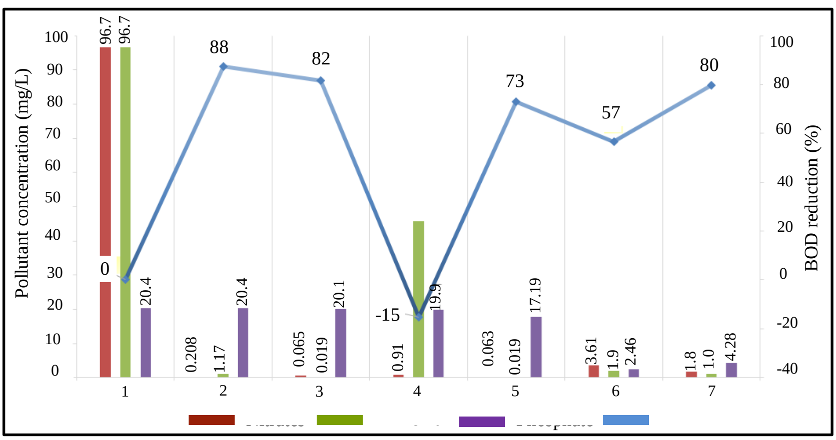


Figure 4. Trends in wastewater pollutant concentration and BOD reduction over time; the numbers in the Y axis refer to the sampling dates indicated in Table

## 4. Conclusion and Recommendation

The results from this study demonstrated that chitin containing microbes have shown a great impact on the reduction of $BOD_5$ of the effluent of wastewater. This reduction could mean a chance of preserving our rivers and reefs which become the recipient of such voluminous wastes. A $BOD_5$ value of less than 20 mg/l was easily achieved with the use of such effective microbes. The advantages of using chitin containing microbes are their cost-effectiveness and environmental friendliness. Depending on the application, microbes have different forms such as loose balls, soil balls, mud balls or a liquid solution. Chitin and microbes have been studied for soil and foliar application in horticulture as an alternative to more sustainable organic agriculture that does not require fertilizers or pesticides.

For broader implementation, a detailed cost-benefit analysis and long-term environmental monitoring are essential to evaluate the economic feasibility and sustained efficacy of chitin-based bioremediation. Proper dosing and the use of standard aeration systems are recommended to maintain the quality of wastewater. Support from policymakers and further research into combined treatment systems could lead to more comprehensive, efficient wastewater treatment practices capable of addressing a wide array of contaminants.

Given the promising results, it is recommended to scale up and integrate chitin-microbe treatments into existing wastewater management systems, particularly in regions with severe nutrient pollution. Optimization of process parameters and dosages should be explored in future research to maximize the pollutant removal efficiency in different environmental conditions.

## 5. Acknowledgement

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