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ORIGINAL RESEARCH ARTICLE

Kinetic modelling for COD and nitrate-N removal from hatchery wastewater through biological approach

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ABSTRACT

This study was conducted to determine the nutrient removal efficiency via the application of mixed cultures from the synthetic hatchery wastewater based on the first-order, second-order and Stover Kincannon models. The synthetic wastewater was prepared according to the characterization of the collected hatchery wastewater sample, and the collected mixed cultures from the pond sediment were acclimatized accordingly. The samples were tested for chemical oxygen demand (COD) and nitrate-N concentration using a Hach spectrophotometer to determine the removal value of the nutrients. The findings show that the highest removal percentage for COD was up to 31.35% on day 3. Meanwhile, the highest removal percentage for nitrate-N was obtained on day 4 at 43.48%. The obtained correlation coefficient, R^2 for COD through first-order, second-order, and Stover Kincannon models is 1, 0.6774, and 0.965, respectively, suggesting that the kinetics of COD removal can be described most properly by the first-order model. A similar model was also reported for nitrate-N with R^2 value of 1, 0.7563, and 0.8693 for the first-order, second-order, and Stover Kincannon models, respectively. Based on the findings, the acclimatized mixed culture used in this study could be a potent natural COD and nitrate-N removal in the hatchery wastewater.

Keywords: hatchery wastewater; kinetic coefficient; COD; nitrate-N; acclimatized mixed culture

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1. Introduction

Aquaculture is one of the most favourable sectors and is rapidly growing globally, supplying 47% of overall human fish consumption[1]. With the growing interest in a protein source and declining catch from the capture fisheries, aquaculture is getting the spotlight from many countries[2]. The hatchery is one of the significant components of aquaculture[3]. During the last few decades, the increasing demand for aquacultural products has led to substantial advancements in aquaculture globally since the application of natural fish supplies is unsustainable[4]. These advancements require more inputs, generating more waste from the production systems. The accumulation of ammonia nitrogen is inevitable since the increase in the intensity of aquaculture is due to the greater use of feeds. Many aquaculture systems produce a large amount of wastewater high in total phosphorus, total nitrogen, organic matter, and suspended solids discharged into the ocean, rivers, and lakes[5]. According to Luo et al.[6], these have triggered an ecological shortcoming through hyper nitrification and eutrophication, which need to be remediated. Also, if left untreated or treated inefficiently, more critical environmental problems will arise, significantly threatening the aquatic ecosystem[4,7].

The efficient treatment of wastewater and discharge management policy for the nitrogenous compounds are obligatory for a sustainable hatchery system and water supplies. Among the various methods currently implemented to treat hatchery wastewater, the biological approach is preferable over chemical and physical techniques for the small capital and running costs, environmental friendliness, low energy use, and not to mention public acceptance[1]. This type of treatment manipulates the ability of microorganisms to convert the suspended organic material into thick biomass, which can be parted by the sedimentation method from the treated wastewater[8]. The microorganisms may be of a single culture or a mixture of several species, forming a consortium. A consortium is a combination of bacterial isolates with the potential to degrade the elements in the wastewater[9]. Due to its ability to degrade a broader range of pollutants, microbial consortia are generally preferred over a single microorganism[10]. The use of single isolates may not be sufficient for the treatment. As elucidated by Dhall et al.[9], a combination of microorganisms can create an accumulative impact on boosting enzyme production, growth productivity, and biomass activity. Also, mixed cultures help overwhelm catabolic repression and feedback regulation, as the products of one culture may serve as a substrate for another. The benefits of the application of microbial consortium include decreasing nitrate, nitrite, ammonia, and phosphorus levels in the wastewater, increasing dissolved oxygen concentrations and promoting organic matter decomposition[11].

The biological treatment of hatchery wastewater can be performed using bacteria, fungi, and algae. Still, the most common one is employing bacteria, which incorporates aerobic and anaerobic treatment. The microorganisms remove the dissolved organic accumulation by degrading it into simpler components during treatment[8]. Biological treatment grants an economical option over chemical and physical treatment methods. It is the most generally adopted method for removing and stabilizing biologically degradable elements in wastewater. The primary advantage of this mixed culture system is that all the nutrients were efficiently removed in a single-stage process[8].

Mathematical models are instruments used to interpret and stimulate the performance of the bioreactor[12], which manage the experimental data with the ease of controlling and monitoring the processes[13]. The mathematical models can also be used to determine the parameters that control the substrate removal rates, optimization and scaling up the bioreactors[14]. Various models, such as first-order, second-order, and Stover Kincannon models, have been widely used to describe the performance of wastewater treatment. These models are the most common kinetic models to determine the substrate removal rate based on influent substrate concentration[12,13]. The Stover Kincannon model is useful in modelling continuous and semi-continuous bioreactors[14]. Also, this model is most commonly used to determine the substrate removal rate at the steady-state conditions[15]. The Stover Kincannon model can be used to describe the relationship between substrate utilization rate and organic loading rate for a bioreactor. The performance of the bioreactor can be assessed based on the kinetic constants obtained from the model[16].

Kinetic studies on the removal of nutrients from wastewater have been of much interest to other countries such as Europe, China and the United States with numerous documentations. However, to our knowledge, Malaysia is still far behind, with few reports available for reference, particularly in wastewater management. Moreover, several researchers have developed numerous unstructured kinetic models to characterize the influence of principle state variables on microbial behaviour. However, there are just a few investigations on the kinetic of nutrient removal employing mixed culture. The most recent study on the removal of nutrients from biorefinery effluent was reported by Jagaba et al.[17], which uses lignocellulosic biomass waste as a biosorbent. Therefore, this study was carried out to evaluate the efficiency of the mixed cultures in the removal of COD and nitrate-N from the synthetic hatchery wastewater by employing the first-order, second-order and Stover-Kincannon models. In this study, the synthetic wastewater was prepared according to the hatchery wastewater sample and was mixed with the mixed cultures to observe the removal efficiency of COD and

nitrate-N from the system. The removal percentage was then calculated based on the initial influent and effluent concentration. The kinetics of the nutrient removal were then determined through the first-order, second-order, and Stover Kincannon models. The most applicable model for nutrient removals is chosen based on the most significant coefficient correlation, R^2 with the value closest to 1. The determination of the kinetic models and their corresponding kinetic coefficients for nutrient removals obtained in this study could be useful in scaling up the bioreactor process performance and gaining better control of the overall process. The influence of waste concentration on these activities may also be determined to assess the mixed culture development. The model of mixed culture development and chemical oxygen demand (COD) and nitrate-N can be derived.

2. Materials and method

2.1. Wastewater collection and preparation

The wastewater used in this study was collected from the hatchery pond. Meanwhile, the mixed culture was sampled from the pond sediment. The collected wastewater was used as a design basis to create the synthetic wastewater. Synthetic wastewater was used throughout the study to avoid further degradation of the hatchery wastewater by the microbial and to standardize the concentration to minimize experimental errors[18,19]. The synthetic wastewater was formulated with a similar composition to the collected hatchery wastewater, with 250 mg/L of COD and 10 mg/L of nitrate-N concentration. In this process, 1000 mL of synthetic wastewater was produced and mixed with 1.4 g fish food pellet solution or commercial nutrient stock (CNS) and 1000 mL of tap water. The solution was then autoclaved at 121 °C for 15 min. 250 mL of the prepared synthetic wastewater was used to feed the mixed cultures daily for 20 days.

2.2. Acclimatization of cultures

Acclimatization of the mixed cultures took place in a bioreactor filled with 3750 mL nutrient stock and 1250 mL mixed culture. The total solid and COD concentration was determined. This is to ensure the complete adaptability of the cultures to the COD content in the sample[20].

2.3. Setup for kinetic assessment

The conical flasks with the same concentrations of AMC and synthetic wastewater were filled to a total volume of 150 mL in ten replicates and were kept at room temperature. The COD and nitrate-N concentrations were determined in the samples using a DR900 Hach spectrophotometer.

2.4. Determination of COD

The COD test was performed to identify the initial and final readings to determine the COD removal value from the wastewater. The initial COD reading on the 0th day was used to identify the initial COD value in all ten flasks. The final reading was obtained on the last day of the experiment. The COD vials were mixed with 2 ml of the 0-th day samples. The blank was prepared by adding 2 ml of deionized water into the COD vial. Both samples and blank vials were heated at 150 °C in a DRB200 reactor for 120 minutes. The COD reading was performed using a Hach spectrophotometer in triplicates to obtain an average value for COD[21,22].

2.5. Determination of nitrate-N

The nitrate-N test was carried out to determine the initial and final readings of nitrate-N concentrations to obtain its removal value from the wastewater. Similar to the COD test, the 0th-day sample was used to determine the initial nitrate-N concentrations in all ten flasks. The final reading was obtained on the last day of the experiment. The sample with a 10 mL volume was added to the vials containing NitraVer 5 nitrate reagent pillow. Meanwhile, the blank was prepared by mixing 10 mL of deionized water and the reagent pillow. Both vials were tested for concentrations using a Hach spectrophotometer[21,23,24].

2.6. Kinetic modelling

2.6.1. First-order nutrient removal model

The rate of change in nutrient concentration, assuming the first-order model for nutrient removal, can be expressed as Equation (1).

$$-\frac{dS}{dt} = \frac{QS_0}{V} - \frac{QS}{V} - k_1 S \quad (1)$$

The rate of change in nutrient concentration due to accumulation ($-dS/dt$) is insignificant; therefore, Equation (1) can be modified into Equation (2).

$$\frac{S_0 - S}{HRT} = k_1 S \quad (2)$$

where S_0 is the influent substrate concentration, S is the effluent substrate concentration, k_1 is the first-order substrate removal rate constant, Q is the inflow rate, HRT is the hydraulic retention time, and V is the volume of the bioreactor. The plotting of $(S_o - S)/RT$ against S provides the first-order kinetic constant value, k_1 in which the value is obtained from the slope of the straight line.

2.6.2. Second-order nutrient removal model

Equation (3) expresses the general equation for a second-order model.

$$-\frac{dS}{dt} = k_{2(s)} \cdot \left(\frac{S}{S_0}\right) 2 \quad (3)$$

The integration and linearization of Equation (3) resulted in Equation (4).

$$\frac{S_0 . HRT}{S_0 - S} = HRT + \frac{S_0}{k_{2(s)} X} \quad (4)$$

2.6.3 Stover-Kincannon model

The equation for the Stover-Kincannon model is displayed in Equations (5) and (6).

$$\frac{dS}{dt} = \frac{Q}{V}(S_0 - S) \quad (5)$$

$$\frac{dS}{dt} = \frac{U_{max}(QS_0/V)}{K_B + (QS_0/V)} \quad (6)$$

Substituting ($-dS/dt$) into $V/[Q(S_0\text{-}S)]$ and plotting against V/QS_0, $1/U_{max}$ and a slope of K_B/U_{max} can be obtained and expressed as Equation (7).

$$\frac{V}{Q(S_0 - S)} = \frac{K_B}{U_{max}} \cdot \frac{V}{QS_0} + \frac{1}{U_{max}} \quad (7)$$

where U_{max} is the maximum utilization rate constant, and K_B is the saturation constant.

3. Result and discussion

3.1. Biomass concentration

Figure 1 presents the biomass concentration of the mixed cultures in the samples for 20 days. The biomass concentration was recorded daily during the acclimatization of mixed culture to investigate the growth rate of the cultures. It was seen from the figure that the biomass concentration increases with days. The determination of the biomass concentration is one of the most commonly used methods to monitor the growth and proliferation of the cultures, providing a simple, reliable and routine means to understand various features of the cultures. The concentration of total solids in the system plays a crucial role in the morphology of the cultures, in which a change in its concentration will lead to a change of morphology since total solids affect the performance of aerobic digestion. Therefore, to increase the efficacy of aerobic digestion, it is essential to recognize the role of the total solid concentration on the behaviour of the microbial communities involved in

aerobic digestion. High total solid contents lead to better aerobic digestion performance.

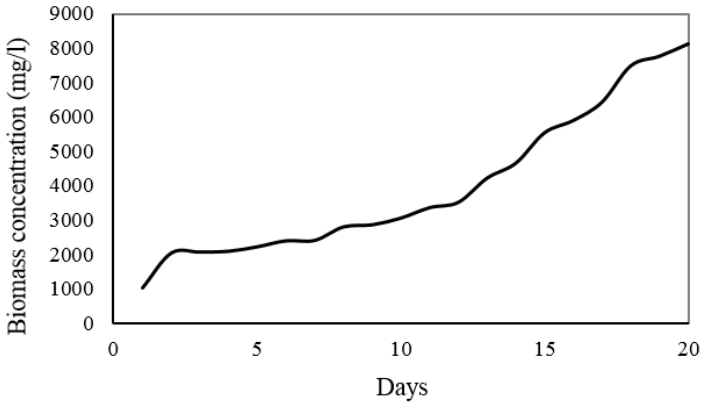

Figure 1. The concentration of biomass recorded for 20 days.

The concentration of biomass across hydraulic retention times is displayed in **Figure 2**. The concentration was seen increasing from 0 to 58 hours, suggesting that the mixed cultures have started to accumulate and undergone the removal of COD and nitrate-N. However, the concentration begins to reduce afterwards, which implies that 58 hours is the optimum time for removing COD and nitrate-N in the wastewater.

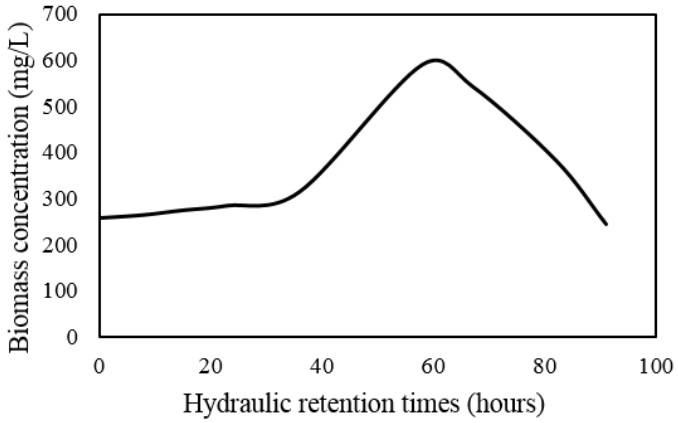

Figure 2. Graph of biomass concentration against hydraulic retention times.

3.2. Nitrate-N analysis

The reading for nitrate-N was recorded every 8 hours for 5 days. **Figure 3** portrays the influence of retention times on nitrate-N removal. As shown in the figure, nitrate-N removal was generally increased with hydraulic retention time and reached its maximum value at 58 hours. Further increase in time reduced the nitrate-N removal. This suggests that 58 hours is the optimum time for removing nitrate-N in the system. Nitrate is an important nutrient required for the growth of the mixed culture, in which the nutrient was used as their food source. Increasing the retention times could promote growth whereby during the growth and development stages, these cultures use and digest more impurities, which helps remove more unwanted nutrients in the wastewater[25]. The removal of nitrate through denitrification involves plenty of oxygen. Hence, as retention time increases, the oxygen amount in the wastewater is reduced, diminishing the removal of nitrate-N in the system[26].

Findings by Lu et al.[26] suggest that 0.7 hours was the optimum hydraulic retention time for nitrate-N removal rather than 12 hours. Also, the activity of nitrate oxidation was remarkably reduced with increasing retention times from 5 to 30 hours. The increase in nitrite-oxidizing bacteria might explain the trend, in which shorter retention times promoted its relative growth, especially the fast-growing *Nitrobacter* sp.[27]. Moreover,

the consortia of *Aeromonas* sp., *Bacillus* sp., and *Pseudomonas* sp. have greatly influenced the total nitrogen removal rather than their respective consortium. The synergistic interaction between these bacterial consortia has been shown to greatly affect the removal of nutrients in the system[28].

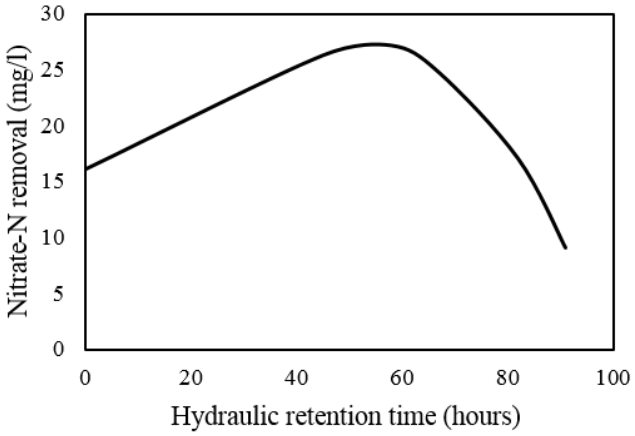

Figure 3. Influence of retention times on nitrate-N concentration.

3.3. COD analysis

Figure 4 presents the effect of retention times on COD removal in hatchery wastewater. The trend shows that the removal value increased over time, suggesting that the COD removal increases as retention times increase. The optimum COD removal was observed at 45 hours; further increase in times reduced the COD removal in the wastewater. Oljira et al.[28] mentioned that the COD removal from brewery effluents increases with the incubation period. Also, similar to total nitrogen removal, the application of three bacterial consortia expedited the removal process compared to the single bacterial application. The reduction of COD concentration is due to the functions of organic materials, which act as a substrate for aerobic microbial metabolism. The finding by Oljira et al.[28] is consistent with the current study, whereby an increase in COD removal was seen with increased retention times, and the application of bacterial consortia showed a significant effect on the removal process.

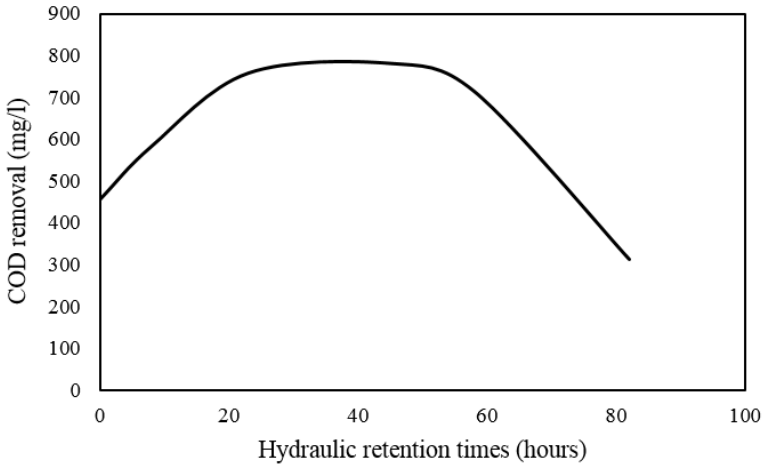

Figure 4. Effect of retention times on COD concentration.

3.4. Kinetics determination

3.4.1. Kinetics of COD removal

The kinetics of COD removal were expressed through first and second-order models as well as the Stover-Kincannon model. **Figure 5** shows the first-order kinetic model for COD removal. The graph was plotted based

on Equation (2), giving rise to the kinetic constant, k_1 value of −0.0063 h^{-1} with the R^2 value of 1. The k_1 value was obtained from the slope of the straight line and is referred to as the first-order kinetic model as the rate of substrate removed by the first-order reaction. The k_1 value, as reported by Akbari et al.[29] and Vandith et al.[15], is 1.882 d^{-1} and 1.01 d^{-1}, respectively. The variation in the k_1 values might be due to the type of wastewater treatment used, wastewater characterization, retention times, and mixed culture communities[15].

Figure 6 displays the second-order kinetic model for COD removal, plotted based on Equation (5), with $k_{2(s)}$ value of −3559.5 h^{-1} and correlation coefficient, R^2 of 0.6774. $k_{2(s)}$ is the speed constant at which the nutrient can be removed from the systems, which suggests that high $k_{2(s)}$ value resulted in the faster nutrients being removed from the systems. The $k_{2(s)}$ will increase as the substrate removal rate increases depending on the initial substrate concentration and mixed cultures communities in the bioreactor. The Stover-Kincannon model for COD removal is presented in **Figure 7**. The maximum utilization rate, U_{max} and saturation constant, K_B values were computed as −27.93 mg/L.hr and 31.96 mg/L.hr, respectively, with R^2 of 0.965. The value of the correlation coefficient R^2 closest to 1 is considered statistically significant. In this case, the first-order and Stover Kincannon models are both significant models, therefore, both models could be used to explain the kinetics of COD removal. Considering the R^2 value for the first-order model of 1, the model is, therefore, more precise in describing the kinetics of COD removal from the hatchery wastewater.

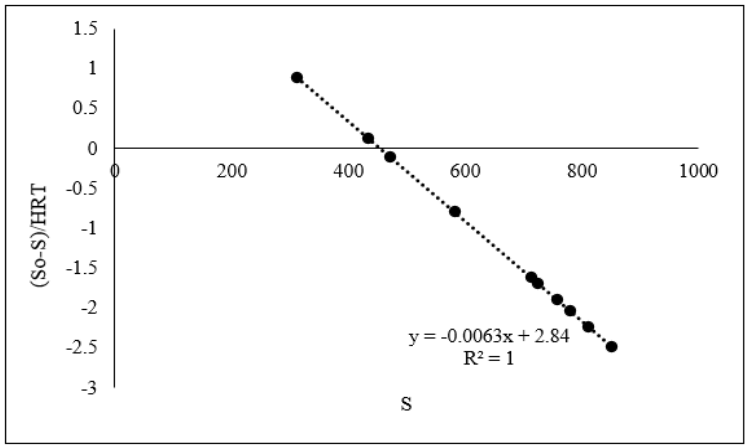

Figure 5. The first-order kinetic model for COD removal.

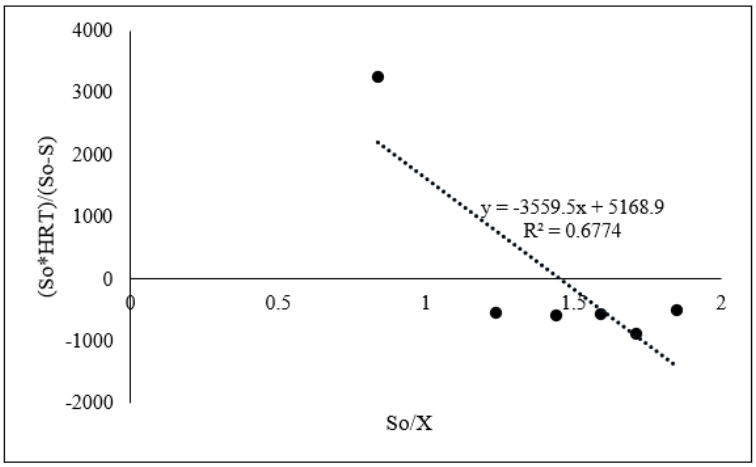

Figure 6. The second-order kinetic model for COD removal.

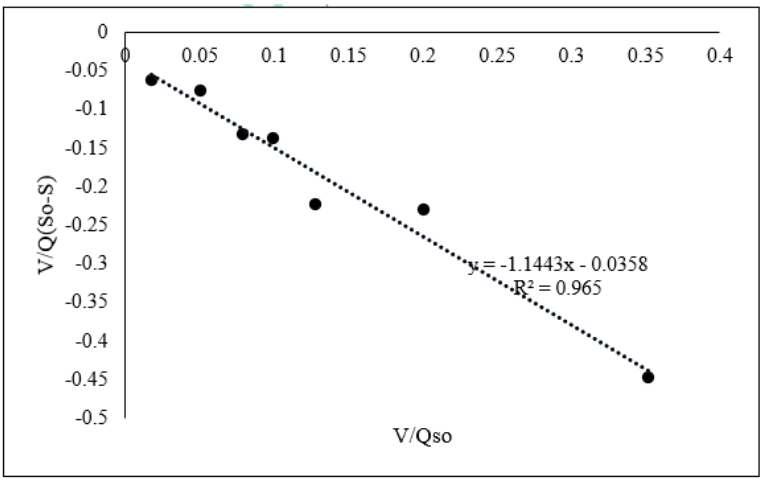

Figure 7. The Stover-Kincannon model for COD removal.

Omil et al.[30] obtained 70%–90% removal of organic matter from the seafood-processing industry. The COD removal efficiency of up to 80% was reported by Guerrero et al.[31] from a fish meal processing factory through anaerobic treatment. The effect of COD concentration, saline wastewater and salt concentrations was investigated by Kapdan and Erten[32] to understand its effect on COD removal. The increase in retention time was seen to improve the COD removal to up to 84%. The Stover-Kincannon model was used to describe the kinetic coefficients, with saturation constant, K_B of 5.3 g/l days, and maximum utilization rate, U_{max} of 7.05 g/l day. Rovirosa et al.[33] investigate saline wastewater treatment through an anaerobic fixed bed reactor. The result shows that to obtain 72% total COD concentration reduction, 24 hours of hydraulic retention times are required. The kinetic constant for COD removal was obtained at 0.679 min^{-1}.

3.4.2. Kinetics of nitrate-N removal

The first-order kinetic model for nitrate-n removal is displayed in **Figure 8**, plotted based on Equation (2), with k_1 value of −0.0063 h^{-1} and correlation coefficient R^2 = 1. Meanwhile, **Figure 9** indicates the second-order kinetic model for COD removal with $k_{2(\text{s})}$ value of 7004.9 and R^2 of 0.7563. **Figure 10** presents the Stover-Kincannon model for nitrate-N removal. The maximum utilization rate, U_{max} and saturation constant, K_B values for the Stover-Kincannon model were calculated as 0.455 mg/l.hr and −0.1753 mg/l.hr, respectively. The k_1, K_B, and U_{max} value obtained in this study was much lower than that obtained by Akbari et al.[29]. This may be due to the variation in the mixed cultures communities and wastewater characterization[17]. Based on the correlation coefficient, R^2 obtained from all models, first-order (R^2 = 1), second-order (R^2 = 0.7563), and Stover Kincannon (R^2 = 0.8793), both first-order and Stover Kincannon models are the two significant models that can represent the kinetics of nitrate-N removal. However, with the value of R^2 equal to 1 for the first-order model, the model is, therefore, the most suited to describe the kinetics of nitrate-N removal.

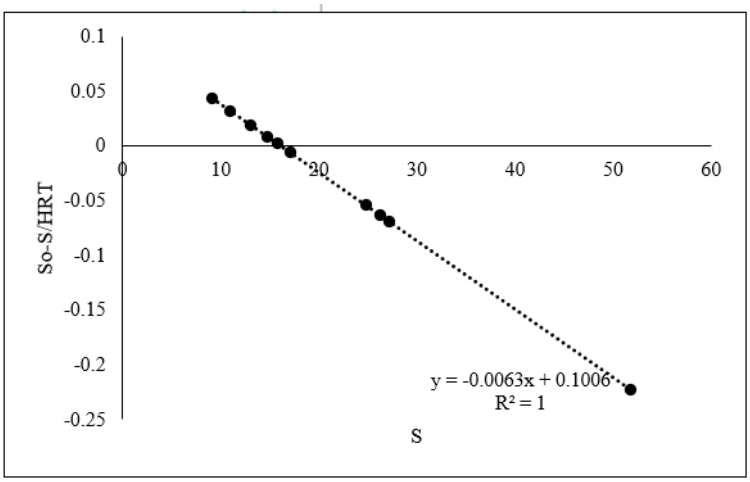

Figure 8. The first-order kinetic model for nitrate-N removal.

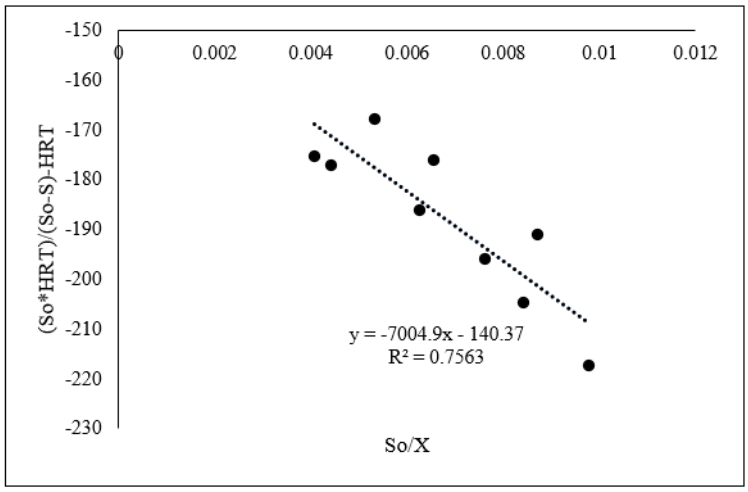

Figure 9. The second-order kinetic model for nitrate-N removal.

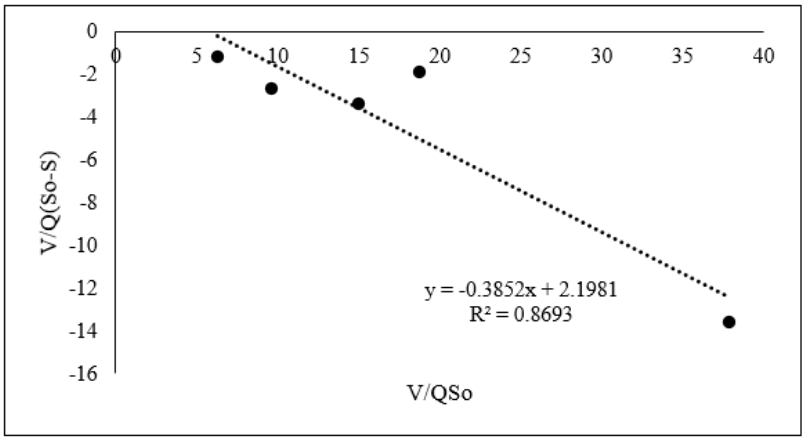

Figure 10. The Stover-Kincannon model for nitrate-N removal.

4. Conclusion

The kinetic constant of the nutrient removal from the hatchery wastewater can be obtained by using three models, which are the first order, second order, and Stover-Kincannon Model. The first-order model was found to be a more appropriate model to predict the removal of COD and nitrate-N in this study. The study shows the potential of mixed cultures in the biological treatment of hatchery wastewater, and the application should be broadened.

Author contributions

Conceptualization, NZ; methodology, NZ; validation, NZ, and NHA; formal analysis, NZ and NHMNR; investigation, NZ and NHMNR; resources, NZ; data curation, NZ, NHA, and NHMNR; writing—original draft preparation, NHMNR; writing—review and editing, NZ and NHA; supervision, NZ; funding acquisition, NZ. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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