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Ammonia-removal potential of a static K2-biofilter media conditioned in mangrove water

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Abstract
This study verified the ability of ammonia-degrading microbes recruited in a static K2 plastic biofilter media in removing ammonia from various samples and in a controlled laboratory condition. Ammonia concentration of about 2.44mg/l was removed by filtering 850 ml ammonia water in 0.43 m^2 biofilter area. This amount of ammonia removal is higher than in normal aquaculture conditions because there is no continuous source of ammonia like those contributed by cultured organisms, feeds, and heterotrophic microbial metabolism. The primary objective of this study is to simply show evidence of ammonia removal by microbes recruited in the plastic biofilter media when other factors that may influence the results were not present. Such complex factors are well-pointed out in the works of other investigators who investigated large facility biofiltration mechanisms. Computing the area of the biofilter media used lead to recommendations on enhancing the existing design (s) and to exceed the benchmark value established in this study.

Keywords: Ammonia-removal, MBBR carrier media, mangrove microbes, wastewater

Introduction
Small-scale Recirculating Aquaculture Systems (RAS) used to grow tilapia and catfish is becoming popular in the Philippines. It was already established that water-quality issues especially ammonia accumulation must be controlled for RAS to be successful. The use of Moving-Bed Biofilm Reactor Filter (MBBR) media is both effective and practical due to its high surface area design and availability in the market. MBBR was found out to be more effective than conventional bio filtration systems such as Trickling Filter, Activated Sludge, RBC, etc. (Chan *et al*., 2009) [3]. Some papers focused on MBBR application in wastewater treatment (Hosseini & Borghei, 2005; Bakke *et al*., 2017) [8, 1] for extraction of unwanted organic substrates and removal of nitrogenous wastes. In theory, the plastic carrier in these systems function only as ammonia filters when they have recruited bacterial populations that are responsible in metabolizing this toxic protein by-product. Recruited nitrifying bacteria usually convert dissolved nitrogenous waste into energy. Ammonia oxidizers that can reduce ammonia nitrogen from the culture water have potential uses in RAS. Some studies noted a few challenges in maintaining their integrity and distribution in biofilters though (eg. Kasmuri & Lovitt, 2018; Erna *et al*., 2013) [10, 6]. Ammonia, nitrite and nitrate even in small concentrations can still cause mortality in fish (Kucuk, 2014) [11]. Activated biofilter media such as in MBBR systems are thus, utilized to maintain water health conducive to larvae and juveniles (Pedreira *et al*., 2014) [16]. The same mechanisms are utilized in RAS (Cahill *et al*., 2010) [2] to provide usable reclaimed culture water, enhanced waste pollutant management and nutrient recovery (Martins *et al*., 2010) [13].
Microbial diversity in mangrove waters has potential in providing biotechnologically useful species (Thatoi *et al*., 2012) [21]. It can contain a diverse group of heterotrophic and ammonia-metabolizing archaea and bacteria that can be recruited in biofilter media biofilms. In RAS, the primary concern is on ammonia removal and microbes involved in this are usually microaerophiles (Ward, 2009) [23]. MBBR systems can be aerated or anoxic, suggesting that ammonia-removal data from various studies can vary. There are no studies that can estimate ammonia-removal in static conditions (no movement/ without aeration) and focusing only on the carrier media in a small-scale setup. Moreover, mangrove-water conditioning has potential in introducing microbial diversity in carrier biofilms and can be expected to enhance its efficiency.

No such study or protocol is available at this time, although this has been perhaps practiced indirectly in many RAS operations when mangroves are tapped as water sources. This simple study was able to get a qualitative snapshot of actual ammonia-removal by mangrove-activated MBBR media (K2) using only an ammonia test kit. In real situations, small-scale RAS farmers rely on this technique in monitoring ammonia build up from excess feed and dead fish decomposition. It is only appropriate that such condition is simulated in the laboratory. This study was able to prove that mangrove microbes can be recruited in MBBR media and removes decomposition ammonia from the water. The surface-to-volume ratio of the plastic carrier was also computed relative to the volume of ammonia water it can process.

Materials and Methods

This study utilized a completely randomized experimental design (CRD), where ammonia water fixed with both conditioned biofilters media and sterile media were replicated three (3) times. Three 5-mL water samples were also obtained from each filtered water for analysis. All the conditions in the control and experimental containers were similar except for the nature of the biofilters medium used. Mangrove water was taken from the mangrove area located within the Iloilo State College of Fisheries Main-Campus, Tiwi, Barotac Nuevo Iloilo, Philippines.

Ten (10) litres of mangrove water were placed in a plastic container to condition three (3) litres of K2 media for 14 days. Low aeration was provided and the biofilters are gently stirred every day. An additional 10 litres of mangrove water serve as backup to replace whatever volume is lost in the conditioning container. This was also aerated and treated with nutrient media.

Fig 1: Plastic biofilters media conditioned in mangrove water

A liquid enrichment media made up of equal parts in volume of

1. F/2 medium for ALGAE,
2. 10% urine solution (10ml urine diluted up to 100ml),
3. Brown sugar (10g dissolved in 200ml water),
4. SOIL extract (50g boiled in 200ml water),
5. Algae wafer extract (boil 50g in 200ml water) was made.

About 10 ml is added to the conditioning container every day. This is to ensure that the nutrients in the water are not depleted to support the microbial community present.

The biofilter media used in this study is made up of HDPE plastic with a size of 7mm x 10mm. The surface area of each biofilter was approximated to the minimum level without considering the corrugations which is already hard to measure. Only the 10mm diameter, 5mm radius, and 7mm height was used to calculate the area of the wheel's circumference and the area of each of the five flat spokes- all are multiplied by 2 because the area estimated is two-sided. The surface area covered by all the biofilters within the volume they occupy was used to estimate the surface-to-volume ratio. This provided hint as to the size of the area that can be covered by the biofilters to support the findings of this study.

Fig 2: MBBR K2 biofilters media purchased online

Ammonia water for the experiments were prepared by placing dead clams in tap water adjusted to 15-ppt salinity by adding table salt. A separate 1-gallon container was filled and refrigerated while waiting for the experiment proper within a 7-day period. Prior to the experiment, the stock ammonia water was also tested qualitatively using a test kit to be able to determine the starting ammonia concentration before introducing the biofilters.

Fig 3: Ammonia was recovered from dead clams placed in the water.

The number of biofilters that can be contained in a 1-liter plastic container was also determined by manual counting. Then, water was added to pre-determine the volume occupied by the biofilters relative to the water that must be added to the experimental containers during the experiment proper. The total surface area was thus measured by multiplying the approximated surface area of each biofilters media to 750. The volume of all the biofilter media was based on the amount of water displaced.

Fig 4: 750 pieces of biofilters media in a 1L container

Water was added in a controlled manner to the 1-liter container filled with biofilters media until it reaches the brim. The amount of water added was subtracted from 1000 ml to get the volume occupied by the biofilters. It is important to note that the biofilters are hollow so a lesser volume occupied was expected. Three (3) 1-liter plastic jars were filled with mangrove-conditioned biofilters media, filled to the brim with aerated ammonia water, and covered for 24-hrs. The same number of plastic jars were filled with sterile biofilters (not conditioned). All were left standing for 24 hours to let the biofilters remove ammonia from the water.

Fig 5: T1-T3 with mangrove-conditioned biofilters; C1-C2 with sterile biofilters.

Three (3) 5-ml water samples were recovered from each experimental container and placed in 10-ml plastic tubes. Each were tested for ammonia levels remaining using an Ammonia Test kit. The color chart shows equivalents in mg/L, but when color fall in between two values, the average is recorded.

Fig 6: Ammonia test kit

The stock ammonia water was also sampled 9 times and tested to ensure that there is no change in the ammonia content during the course of the 24-hr experiment. Also, due to the nature of the data which rely only on color grading to estimate the ammonia content, a non-parametric Kruskal-Wallis test at 0.05 level of significance was used to compare the ammonia content among the stock ammonia water samples, samples from water with sterile biofilters, and samples treated with mangrove-conditioned biofilters. Independent samples T-test at 0.05 level of significance was used for post-hoc analysis.

Data and Results

An initial look at the test tubes showed that ammonia was removed from the water when biofilters conditioned in mangrove water was used.

Fig 7: Ammonia test results using sterile (C1-C3) biofilter media and conditioned (T1-T3) biofilter media.

Based on the color reaction, there is almost no (yellow = 0.0 mg/L Ammonia) more ammonia left in T1 to T3 tubes. On the other hand, ammonia is still present in C1-C3 tubes which was only left with sterile biofilters. The same color reaction was observed in 9 samples of stock ammonia water suggesting that ammonia concentration did not change during the 24-hr experimental period.

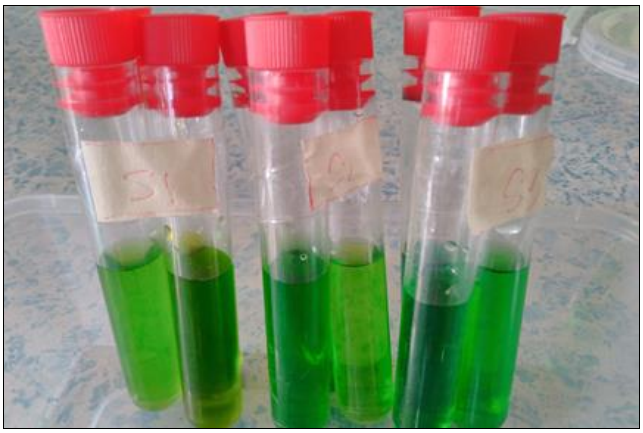

Fig 8: Ammonia test results of stock ammonia solution.

Kruskal-Wallis test at 0.05 level of significance showed that there are significant differences ($p=0.00$, $\alpha= 0.05$) among the samples from three water sources in terms of ammonia levels.

Table 1: SPSS Output on comparing mean ranks of ammonia levels among 3 sample sources.

Hypothesis Test Summary				
S. No	**Null Hypothesis**	**Test**	**Sig.**	**Decision**
1	The distribution of ammonia Present is the same across categories of Sample source	Independent sample Kruskal- Walls Test	.000	Reject the null hypothesis

Asymptotic significance are displayed. The significance level is .05

Since there is no fixed post-hoc analysis in SPSS for non-parametric tests, data was transformed to mean ranks to be consistent with Kruskal-Wallis treatment of data and analyzed using independent-samples T-test at 0.05 level of significance to determine which among the 3 sample sources is significantly lower in terms of ammonia levels. Water treated with conditioned biofilters have significantly lower ammonia levels (mean= 0.11 mg/L) compared to water coming from the stock ammonia solution (mean= 2.28 mg/L; p=0.003) or ammonia water treated with sterile biofilters (mean= 2.44 mg/L; p=0.002). There is no significant difference between the stock ammonia water and water treated with sterile biofilters (p= 0.73).

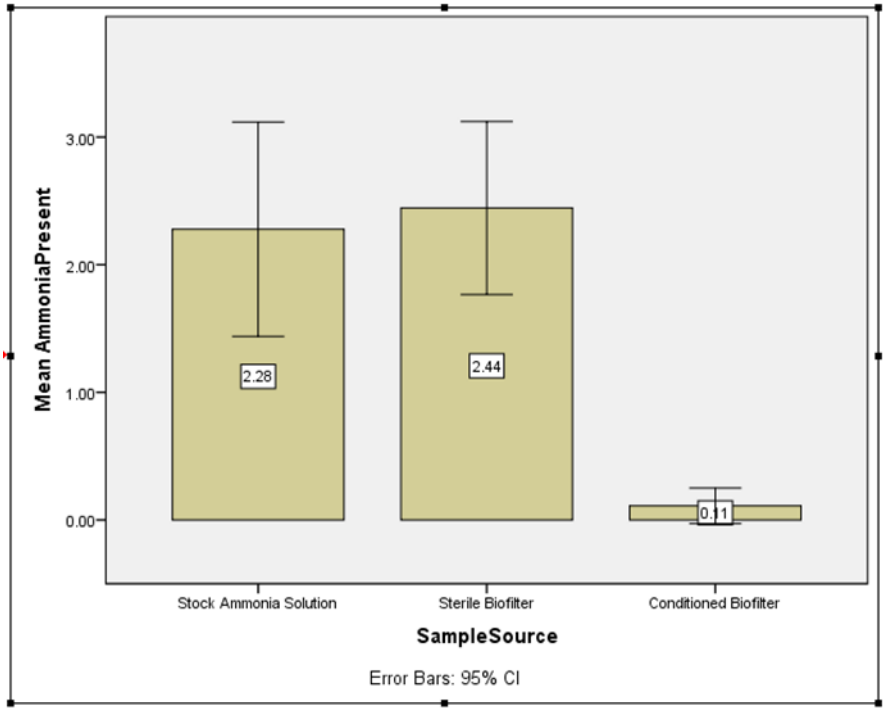

Fig 9: Bar chart showing ammonia level means from different water sources, with error bars at 0.05 level of significance.

The surface area of the biofilter was computed using the specified dimensions below.
Area of rim = 31.42mm^2 x 7mm^2 x 2 (sides) = 220mm^2
Area of spoke = 5mm^2 x 7mm^2 x 5 (pcs) x 2 (sides) = 350mm^2
Single biofilter area = 220mm^2 + 350mm^2 = 570mm^2
Total area = 570mm^2 x 750pcs = 427,500mm^2 = 0.4275 m^2

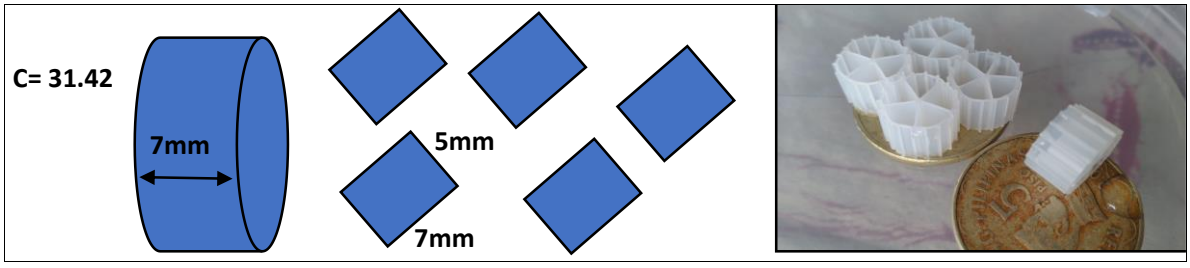

Fig 10: Breakdown of every measurement used in the approximation of the biofilter media surface area.

The total volume of water displaced by the biofilters in a 1-liter experimental container is 1000mL - 850mL (volume of water added up to the brim) = 150mL. The biofilters indeed can provide a filtration surface of 0.4275 M^2 per 0.15L volume of the media.

Surface to Volume Ratio = 0.4275 M^2: 0.15 L
= 2.85 Cubic Meter Filtration Area per Litre Biofilter

In this study the amount of water filtered per biofilter area is equal to (0.85 L / 0.4275 M^2) = 1.99 L/M^2.

Discussion

It is obvious that conditioned biofilters in this study were able to recruit ammonia oxidizers and removed ammonia from the water. There are plenty of studies (eg. Diaz *et al*., 2011, Pedreira *et al*., 2014; Summerfelt, 2015, von Ahnen *et al*.,

2015) [4, 16, 19, 22] quantifying ammonia removal especially in recirculating systems. Their findings came from a complex environment and in a large-scale system though. MBBRs otherwise referred to as "slurry reactors" utilizes suspended and attached microbial biofilms on mobile and suspended carriers inside a bioreactor (Tang *et al*., 2016) [20]. There are no studies to date that investigated ammonia removal using static MBBR media in a 1-liter container. The experiment was able to focus solely on detecting microbial action alone in the ammonia removal process using a color grading diagnostic technique (eg. Ammonia Test Kits) that is also employed by small-scale RAS operators in the Philippines. Thus, other factors that may affect ammonia levels in the water were cut off. This established that whatever amount of ammonia was removed, the researcher can only attribute that to the conditioned biofilter media.

In RAS, Total Ammonia Nitrogen (TAN) is the most important water quality indicator. MBBR media supports slow-growing nitrifying bacteria that can remove TAN (Piculell *et al*., 2016; Young *et al*., 2017) [17, 24]. Nitrification is necessary for water quality maintenance in the RAS (Pedersen, Oosterveld, & Bovbjerg, 2015) [15]. Nitrification converts Total Ammonia Nitrogen (TAN) to nitrate (NO_3-N) under aerobic conditions. Usually, it occurs in two essential stages mediated by two groups of autotrophic nitrifying bacteria in the presence of oxygen. The first stage is the TAN oxidation to nitrite (NO_2-N) (nitritation), which is carried out by the ammonia-oxidizing bacteria (AOB). Next stage is the immediate breakdown of NO_2-N to NO_3-N (Nitritation), performed by the nitrite-oxidizing bacteria (NOB) (Ebeling, Timmons, & Bisogni, 2006; Ge *et al*., 2015) [5, 7]. These nitrifying bacteria utilizes nitrite ions and ammonia molecules as their sole sources of energy for metabolism and cellular growth (Stein & Klotz, 2016) [18]. Nitrifying AOB and NOB are Gram-negative. Previous studies have reported that AOB included the genus *Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosovibrio, and Nitrosolobus*. At the same time, NOB was dominated by the genera of *Nitrococcus, Nitrobacter, Nitrotoga arctica, Nitrolancea hollandica, Nitrospina,* and *Nitrospira moscoviensis* (Ge *et al*., 2015; Lepine, 2018) [7, 12].

To give a simple comparison of the performance of the conditioned biofilters in this study, Huang *et al*. (2018) [9] found that the mean ammonia concentration entering the biofilter tanks in a Recirculating Aquaculture System (RAS) for grouper is 0.219 $\pm$ 0.012 mg/L. This is given that the waters have already been filtered. Nootong and Powtongsook (2012) [14] also have 0.24 ± 0.09 mg/L ammonia in their compact aquaculture system. These values are already considered as "efficient removal". In this study, an even higher concentration of 2.44 mg/L was reduced in 24 hours to almost 0.11 mg/L. Of course, compared to a large-scale RAS, there is no more additional ammonia that was introduced other than that in the stock solution. Moreover, the ratio of the biofilter surface area to the volume of water filtered is relatively higher in this study, because there is almost the same space occupied by the water and the biofilter. In a typical RAS, the biofilter area is less than 5% of the overall components of the system. This means, relatively larger amounts of water are filtered per unit area of the biofilter thus, reducing the ammonia to zero is impossible.

This study was also able to compute the total surface area of the biofilter that treated about 850 mL of ammonia water. Existing studies did not bother to check this. Even specifications of biofilter media sold in online stores do not give such value. In this study however, only the minimum area approximation was determined because of the limitation to compute the area of even minute corrugations present in the surface of the media. All-in-all, the filtration of 1.99 L/M^2 can be a benchmark value that can inform future studies. Computing the surface area can also open more ideas on how to increase it by improving the design. You can easily imagine about 2 liters of water spread over a square meter of biofilter surface. A cubic meter has a thousand liters of water so basically, 2 liters on a square meter of surface is a thin spread. This can support the findings about its efficiency in the experiments.

The stock ammonia solution was expected to maintain the ammonia levels because it is covered and protected from external conditions. On the other hand, in control containers with sterile biofilters, ammonia levels also remain the same despite the idea that it is exposed to air and other unknown factors. This simply suggests that only microbial action via nitrification can remove ammonia content in aqueous environments. It also implies that recirculating aquaculture systems must really rely on microbes in terms of ammonia removal. Providing them with more surface area to thrive and enough nutrients to grow should be the primary goal of every aquaculturist. Perhaps the enrichment procedure of the mangrove water also enhanced the activity of the microbes that were recruited in the biofilters.

Conclusion and Recommendation

This study was able to verify the potential of microbes recruited in plastic biofilter media to efficiently remove ammonia from the water. The performance of the K2 biofilter was also established after the total surface area was computed relative to the amount of water it can filter. Ammonia was also found to be a stubborn toxic chemical that cannot be removed by any practical means except through nitrification. There are still potential follow-up studies that can be carried out by future researchers. It is recommended that a more periodic monitoring (eg. per hour removal/liter/surface area) of ammonia levels using a more accurate analysis instrument must be done. The recruitment process is also an interesting area of investigation. Enhancing colonization of an artificial substance like plastic can contribute more to the overall efficiency and sustainability of the biofilter media. Finally, this preliminary finding calls for innovative ideas focusing on its design to improve the efficiency of ammonia removal relative to its surface area.

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