International Journal of Agricultural Technology 2019 Vol. 15(5):735-746
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The utilization of charcoal from agricultural residual waste materials in nitrification from hybrid catfish cultured water

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Phinyo, M., Kapud, J. and Inyawilert, W. (2019). The utilization of charcoal from agricultural residual waste materials in nitrification from hybrid catfish cultured water. International Journal of Agricultural Technology 15(5):735-746.

Abstract Aquaculture growth activities have increased in high nitrite and nitrate concentration in wastewater. The nitrification of three agricultural residual wastes (i.e., coconut shell, durian peel, and corncob) as materials for fixing bacteria involved in nitrification was reported. Charcoal from microbial curing of three materials reduced the amount of TAN (Total ammonia nitrogen) in hybrid catfish cultured water from TAN from greater than 4 mg-N/L to 0.5 mg-N/L by transformation to nitrite and nitrate. Nitrification was completed within three days, with all three charcoal types reducing ammonia at similar levels. While the control set (without the charcoal), TAN concentrations were at least 3 mg-N/L, with minor accumulations of nitrite and nitrate accumulation.

Keywords: agricultural charcoal, nitrification, total ammonia nitrogen

Introduction

The growth of the aquaculture industry (includes aquaculture species except for aquatic plants) in Thailand has reached 934,758 tonnes, equivalent to $2,636 million USD. In 2014, Thailand was ranked in the top 10 aquaculture industries in the world, and is likely to increase, especially in catfish cultivation, which accounted up to 86,475 tonnes, or 30%, of total production (FAO, 2017 and 2018).

The expansion of the aquaculture industry has greatly impacted aquatic ecosystems through unconsumed and indigestible food, water runoff, and wastewater emitted into natural water resources, which contain high levels of nitrites, nitrates, and ammonia from fish urine (Wongkiew *et al.*, 2017; Timmons *et al.*, 2002). These increase water toxicity, ammonia is highly toxic to aquatic organisms, affecting the liver, kidneys, blood system, gill epithelium, oxygen exchange, and osmoregulatory activity. It also reduces immunity

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resulting in a higher chance of infection from microorganisms (Camargo and Alonso, 2006; Augspurger *et al*., 2003; Paerl, 1997; Russo, 1985).

Nitrites can be found in two forms: ionized (NO_2-) and non-ionized (HNO_2). Non-ionized nitrites will reduce nitrification, which is toxic to bacteria such as *Nitrosomonas* spp. and *Nitrobacter* spp. If water pH increases, ionized nitrite will be found more than non-ionized nitrite. In the aquaculture system, NO_2- is more abundant than HNO_2, which can cause hypoxia in aquatic organisms. The long-term effects of nitrites on mammals include the formation of nitrosamines in the gastrointestinal tract, resulting in increased cancer risk. Ammonia nitrite treatment can be used to reduce the risks associated with water contamination, utilizing bacteria in each nitrification step (Mook *et al.*, 2012; Camargo and Alonso, 2006; Anthonisen *et al.*, 1976; Lewis and Morris, 1986). Ammonia nitrate treatment involves the conversion of ammonia to nitrites and nitrites to nitrates. The nitrification caused by biological activities relies on the activity of two types of bacteria in the chemoautotrophic bacteria group (i.e., ammonia-oxidizing bacteria; AOB): *Nitrosospira* spp. and *Nitrosomonas* spp (Schramm *et al.*, 1998). These bacteria function in the ammonia oxidation process, converting ammonia to nitrite. Nitrite-oxidizing bacteria (NOB) such as *Nitrospira* spp. and *Nitrobacter* spp., function to transform nitrite into nitrate, which will produce higher accumulations of nitrate in water (Yao and Peng, 2017; Fujitani, *et al.*, 2015; Mari *et al*., 2012; Purkhold *et al.*, 2000; Arciero *et al.*, 1991; Hooper *et al.*, 1997).

In 2016, production yields of durian, coconut, and corn across Thailand were 0.66, 0.86, and 4.06 million tonnes per year, respectively (Office of Agricultural Economics (OAE); 2017). Numerous residual materials from the agricultural sector, including durian bark, coconut shell, and corncobs, are discarded, which contribute to environmental pollution. However, these materials have the potential to be utilized as energy sources for biomass fuel (Bogale, 2009). The charcoal obtained from these agricultural materials has a different surface characteristic. Therefore, it is of interest to utilize charcoal from these agricultural materials as an intermediate of bacteria in the nitrification. The purpose of this study was to investigate nitrification in agricultural residual wastes as material for fixing bacteria involved in nitrification to reduce ammonia in catfish culture.

Materials and Methods

Preparation of charcoal from agricultural residual wastes

Agricultural residual wastes, including durian bark, and coconut shell, were collected from local markets, and corncobs were obtained from a corn seed removing factory in Phitsanulok, Thailand. The materials were washed with distilled water and baked at 80°C. The materials were burned in a 200 L oil barrel for approximately 1-2 h and oven dried at 80°C for 24 h before being characterized by Scanning Electron Microscope (SEM) model JSM-6610 LV.

Fixing natural bacteria by using charcoal and testing the nitrification

The three charcoal types were shredded into 1 cm pieces and sieved. Charcoal was then packed in a bottle with an 8 cm diameter and height of 15 cm. The coconut shell, durian shell and corncob charcoal samples were weighted as 245, 240, and 330 g, respectively. Charcoal was packed in three mesh bags and incubated with 300 L catfish cultured water in a 500 L fiberglass tank for 14 days to obtain nitrifying bacteria. The water was changed and given 20 g of commercial fish feed (30% protein) for 21 days while the air was added throughout the incubation period (Malaphol, 2008). The charcoal bag was then placed in a 40 * 60 * 30 cm (W * L * H) glass cabinet, enclosed with black plastic. In total, four sets of experiments (without charcoal as the control and 3 type of charcoal for experiment), each repeated three times, were performed to test nitrification using 8 mg-N/L of NH_4Cl 40-L per cabinet. Batch experiment was conducted three times, starting when the materials were 45, 49, and 53 days old. The parameters for water quality were analyzed every day. Ammonium, nitrite were measured by the colorimetric method, and nitrate was analyzed using the cadmium reduction method (Strickland and Parson, 1972). The dissolved oxygen (DO) and temperature were measured using HANNA model HI 9147 (measured every 6 h).

TAN removal and water quality by using charcoal in catfish cultured water

Hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) were reared until they reached an average body weight of 20 g. Density of fish was 60 fish/m^3 in a 500-L fiberglass tank. Fish were fed on a commercial diet two times a day for 7-9 days. Ammonia concentrations were measured at 4 mg-N/L. Following this reading, water was placed into a 40-L glass cabinet containing three types of 96 day old charcoal samples. During the experiment, air was filtered and water was analyzed every day. Ammonia, nitrites, nitrates, DO, pH (measured by pH meter), temperature, alkalinity (Alkaline test kit, VBC, Thailand), chlorophyll-a (Strickland and Parson, 1972), and Total suspended solids (TSS) (Boyd and Tucker, 1992) were recorded. Tests were conducted three times, using cultured water with incubated charcoal.

Results

Morphology study of charcoal from agricultural residual wastes

Coconut shell charcoal (A) had a moderate porous surface, while the durian bark charcoal (B) had a structure arranged in a layer similar to bark with a low porous surface. Corncob charcoal (C) had rough, high porosity, and high complexity surface (Figure 1).

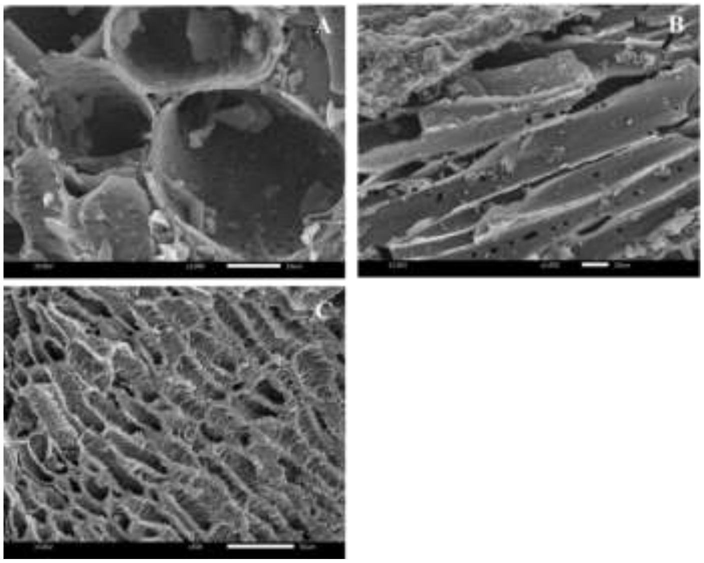

Figure 1. The coconut shell charcoal structure (A) the durian bark charcoal structure (B) the corncob charcoal structure (C) under SEM

Nitrification in charcoal using ammonium chloride solution

The nitrification test of the charcoal samples at 45, 49, and 53 days showed that the level of ammonia chloride decreased from 8 mg-N/L to less than 0.5 mg-N/L within two days. The coconut shell charcoal yielded the highest reduction of ammonia, accumulating in the form of nitrate up to 3.89 mg-N/L. Nitrite was not observed on the second day of the treatment phase, while in the control cabinet without charcoal on the second day, total ammonia nitrogen (TAN) remained in the system more than 6 mg-N/L. The nitrite and nitrate were low (Figure 2). During the nitrification test, water quality was analyzed. The parameters included temperature, pH value, and dissolved oxygen in water as shown in Table 1.

Table 1. Water parameter during ammonia chloride reduction test at 8 mg-N/L and results of one-way ANOVA statistics (*P*-value)

Parameter	**Average of value ± Standard deviation (minimum-maximum)**				***P*-value**
	Control	**Coconut shell**	**Durian shell**	**Corncob**	
Temperature (^{o}C)	28.5±1.4 (26.8-31.8)	28.5±1.6 (26.2-32.3)	28.4±1.4 (26.6+31.5)	28.4±1.6 (26.4-32.3)	1.00
pH	8.28±0.14 (8.01-8.55)	8.26±0.16 (8.02-8.50)	8.26±0.19 (8.01-8.65)	8.22±0.33 (7.11-8.51)	0.67
DO (mg/L)	6.88±0.30 (5.95-6.93)	7.01±0.37 (6.33-7.45)	6.81±0.49 (6.05-7.68)	6.96±0.44 (6.13-7.60)	0.77

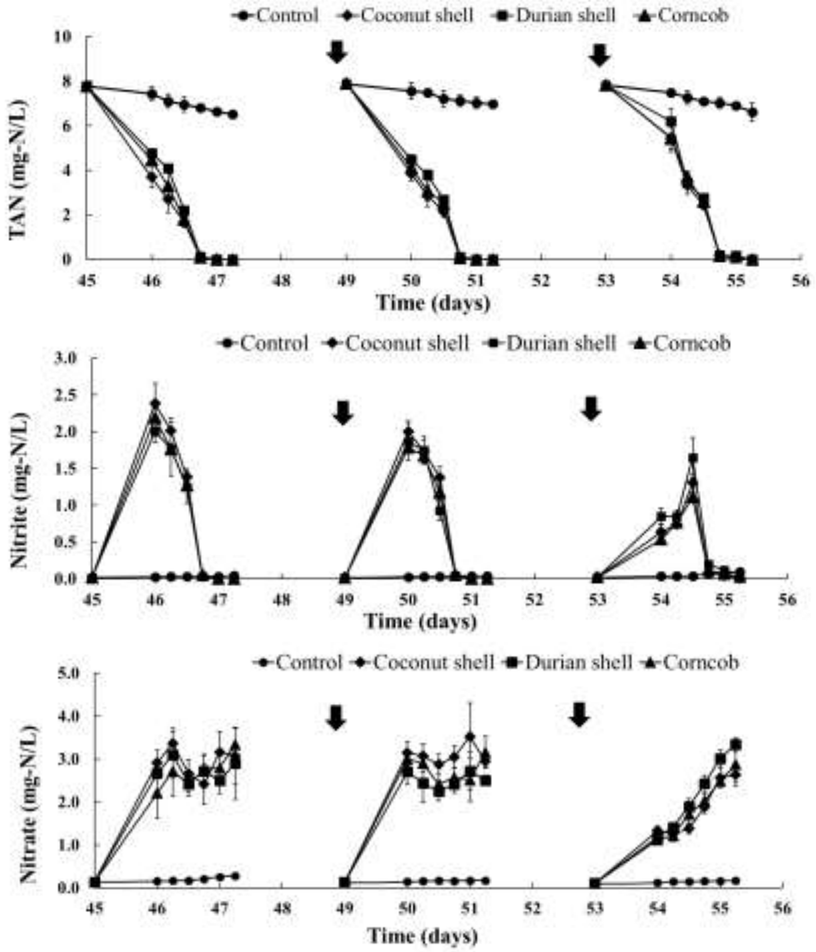

Figure 2. Total ammonia nitrogen (TAN), nitrite and nitrate level in nitrification utilizing ammonia chloride solution 8 mg-N/L

Nitrification in charcoal using hybrid catfish cultured water

TAN values were reduced to less than 0.5 mg-N/L within three days where the reductions of TAN values for each charcoal type (i.e., coconut shell, durian peel, and corncob) were in the same range. When compared to the control group after three days, TAN values remained constant at more than 3 mg-N/L. The amount of nitrite in each experiment set was limited to 3 mg-N/L and changed to the highest cumulative nitrate value at 4 mg-N/L (Figure 3). In each period, the temperature was between 26℃-32℃. The average pH was 7.88-7.94. The oxygen levels in the water were not less than 6 mg/L, and the alkalinity values were between 103.3-121.0 mg $CaCO_3$/L. The suspended solids were 116.9-125.7 mg/L and chlorophyll-a values were low and found to be similar in each batch. The results of one-way ANOVA statistics indicated that the water parameter were no significant difference ($P>0.05$) (Table 2).

Table 2. The water quality during the nitrification test in hybrid catfish cultured water and results of one-way ANOVA statistics (*P*-value)

Parameters	**Average of value ± Standard deviation (minimum-maximum)**				***P*-value**
	Control	**Coconut shell**	**Durian shell**	**Corncob**	
Temperature (^oC)	28.2±2.4 (26.1-31.8)	28.4±1.9 (26.0-31.6)	28.5±2.0 (25.6+32.0)	28.5±1.9 (26.1-31.9)	1.00
pH	7.88±0.3 (7.10-8.39)	7.93±0.27 (7.40-8.41)	7.92±0.22 (7.45-8.39)	7.94±0.31 (7.00-8.51)	0.66
DO (mg/L)	6.42±0.21 (5.95-6.93)	6.48±0.29 (5.65-6.98)	6.53±0.26 (5.90-7.00)	6.42±0.36 (5.85-7.03)	0.32
Alkalinity (mg $CaCO_3$/L)	121.0±20.4 (160.7-86.7)	103.3±25.6 (147.5-67.5)	104.2±27.0 (147.5-65.0)	103.3±27.5 (145.0-60.0)	0.22
Total suspended solid; TSS (mg/L)	119.0±14.1 (106.0-134.0)	125.7±31.1 (90.0-147.0)	116.9±10.3 (110.0-128.8)	117.2±17.9 (98.3-133.8)	0.94
Chlorophyll a (mg/m^3)	0.04±0.05 (0-0.09)	0.05±0.03 (0.02-0.08)	0.02±0.02 (0-0.02)	0.04±0.07 (0.01-0.16)	0.80

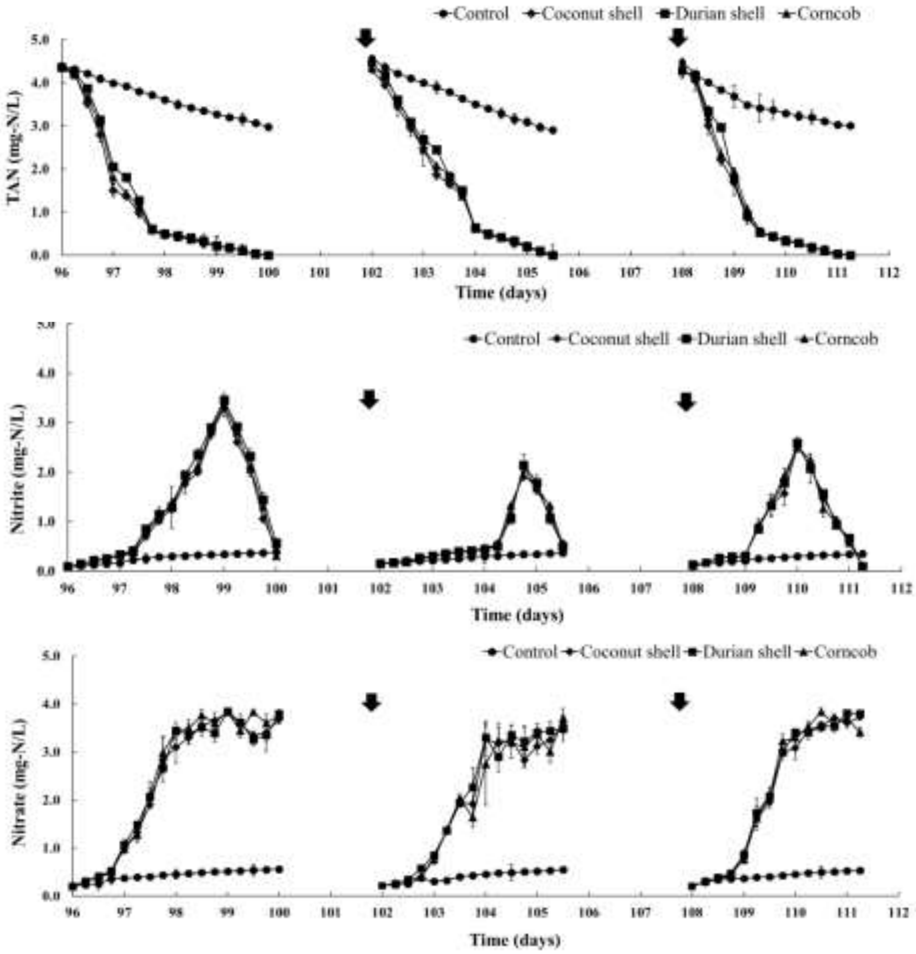

Figure 3. Total ammonia nitrogen (TAN), nitrite and nitrate of hybrid catfish uncultured water utilizing charcoal

Discussion

From the study of the charcoal characteristics using scanning electron microscope (SEM), corncob charcoal was shown to have the highest area of porosity, followed by coconut shell charcoal and durian shell charcoal. A large surface area is necessary to increase the area of adhesion for bacteria to have an effect on the nitrification rate (Kugaprasatham *et al.,* 1992). The structure of charcoal depends on the composition and arrangement of each type of material, as well as heat and burning time (Tripathi *et al.,* 2016; Mokhtar *et al.,* 2012; Chiang *et al.,* 2001). Corncob had the largest surface area, followed by coconut shell charcoal and durian shell charcoal, equal to 160, 36.5, and 3.35 m^2/g

(Songrit *et al.*, 2017; Iqbaldin *et al.*, 2013; Sun and Webley, 2010). From the research regarding the burning of the coconut shell charcoal at varying temperatures (532 ℃, 700 ℃, and 868 ℃), it was observed that when using higher heat, the material will be less porous (Budi *et al.*, 2013). The surface and pore area are also related to the adsorption process of charcoal. The higher surface area and higher porosity of charcoal, the higher the adsorption properties (Mangun *et al.*, 2001). The method of increasing porosity is activated carbon which can be conducted in several ways, such as using chemicals (KOH and NaOH) or different heating methods (Mook, *et al.*, 2012; Foo and Hameed, 2012; Monsalvo *et al*., 2011; Mokhtar *et al.*, 2013).

It was observed that when treated with ammonium chloride (NH_4Cl), TAN contents of the three types of charcoal and catfish cultured water were reduced. The ammonia level can be reduced to less than 0.5 mg-N/L within two to three days, and transformed to nitrate. The nitrite can also be transformed into higher nitrates. In the system, complete nitrification occurred. Compared with the control experiment, there was a slight TAN reduction, with transformation to nitrites and nitrates, showing there was no nitrification in the control cabinet without charcoal. During the experiment, a small amount of chlorophyll-a was produced by nitrification, mainly caused by the activity of nitrifying bacteria. In addition, all three types of agricultural residual wastes have intermediate properties that can cause the bacteria in nitrifying groups to bind to the surface area. This is similar to previous research which utilized wood powder as an intermediate to immobilized nitrifying bacteria to reduce the amount of TAN in the black tiger shrimp (*Penaeus monodon*) culture system. It was observed that the amount of TAN can be reduced at a rate of 2.4-2.7 mg/L in 20 L water within three days (Manju *et al.*, 2009). Furthermore, the study showed that charcoal can absorb nutrients from the soil. It was observed that *Nitrosospira* sp. (AOB group), caused increased nitrification when adding charcoal to the ground of selway-bitterroot wilderness in Northern Idaho (Ball *et al.*, 2010). This corresponds with research that used coal in the Eastern Sierra Nevada forest area, and found that microbial respiration and nitrification increased (Carter *et al.*, 2018). Furthermore, the ratio of microorganisms associated with nitrification is also related to the rate of reaction. AOB bacteria must be higher than NOB in nitrification (2:1, AOB:NOB), as the rate of ammonia to nitrite transformation must be higher than that of nitrate (Fujitani, *et al*., 2015; Purkholdetal., 2000; Yao and Peng, 2017; Arciero *et al.*, 1991; Hooper *et al.*, 1997; Mari *et al.*, 2012). In addition, DO is related to nitrification, since nitrifying bacteria require oxygen (Hu *et al*., 2005; Stenstrom and Poduska, 1980). Oxygen will affect the function of AOB bacteria if dissolved oxygen (DO) values in the system are less than 4 mg/L. The activity of AOB in reducing ammonia will also decrease and the NOB bacteria will be less functional when DO values are less than 2 mg/L (Wongkiew *et al.,* 2017; Kim *et al.,* 2005). Water temperature also affects the function of AOB and NOB bacteria. The optimum water temperature should be between 25 ℃-30 ℃; any higher and the amount of bacteria will decrease, resulting in lower nitrification (Kinyage and Pedersen, 2016; Baer *et al.,* 2014; Zhang *et al.,* 2014). However, the temperature can exceed 30 ℃ for a short period of daytime and still have efficient bacterial activity. The average pH of the hybrid catfish water during the treatment was 7.88-7.94, and the average alkalinity was 103.3-121.0 mg $CaCO_3$/L where the pH and alkalinity values were related. Previous research observed that the bacteria will function when the pH is greater than 9.0 and below 6.4, which is related to the alkalinity with buffer properties. The alkalinity causes the reduction of nitrification when the alkalinity is less than 100 mg $CaCO_3$/L, while the aquaponic system should have alkalinity between 100-150 mg $CaCO_3$/L. The alkalinity can be balanced by adding KOH, $NaHCO_3$ or $Ca(HCO_3)_2$ into the system (Delong and Losordo, 2012; Ruiz *et al.,* 2003; Wongkiew *et al.,* 2017).

The results of the study revealed that coconut shell charcoal, durian shell charcoal, and corncob charcoal have properties that serve as intermediates for nitrifying bacteria adsorption, which can cause complete nitrification from the treatment of catfish cultured water. The reduction of TAN values in each type of charcoal was comparable. While the corncob charcoal has a higher pore volume, the catfish cultured water contains high level of suspended solids. These suspended solids will be trapped in the pore and reduce the contact between bacteria and water. Therefore, the sediment must be removed by cleaning to reduce clogging.

Acknowledgements

This research was financially supported by Naresuan University (grant number R2561B054) the fiscal year 2018, Faculty of Agriculture, Natural Resources, and Environment, Naresuan University.

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(Received: 23 May 2019, accepted: 28 August 2019)