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Reuse of briny wastewaters from the pickling process of mustard leaves in marine fish culture

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ABSTRACT

This study investigates the re-use of briny wastewater from the mustard leaf pickling process in fish culture. Three brine water samples, including pickling water, water extracted from waste mustard blades, and water extracted from waste mustard stems, were collected and diluted with seawater. Dilution ratios of 10^3–10^4 were sufficient to adjust the characteristics of the three water samples so that they were similar to marine aquaculture environments. Juvenile tilapia fish, *Oreochromis mossambicus*, were raised in the diluted and aerated water samples for 1 month. The results indicated that tilapia cultured in the three diluted water samples exhibited similar average weight gains (13–16%) and specific growth rates (0.66–0.74% d^{-1}) compared to a control sample raised in seawater only. This indicates that briny wastewater from the pickling process of mustard leaves can be used for marine fish culture instead of being treated as an industrial wastewater.

Keywords: Pickling mustard leaves; Briny water; Dilution; Fish culture

1. Introduction

Pickling mustard (*Brassica juncea*) leaf is a traditional side dish in Southeast Asian countries [1]. However, the mustard leaf pickling process requires large amounts of pickling salt and generates a large amount of briny wastewater. In addition to wastewater, large amounts of the salted outer blades and inner stems are cut off and thrown away.

The wastewaters and solids produced in the mustard leaf pickling process are characterized by high salinities, high organic matter contents, high oxygen demands, and low pH, etc. [2–4]. The wastewater must be treated by specific wastewater facilities due to its high salinity and low pH levels, which inhibit the growth of microorganisms [3,5]. Most pickling

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wastewaters are treated by physicochemical methods, including evaporation, condensation, coagulation flocculation, ion exchange, or membrane techniques [3,6]. However, these procedures require a lot of resources or chemicals and involve high facility costs [7]. However, researchers have developed some biological methods to decompose the organic portion of pickling wastewaters. These methods require specific salinity-acclimated microbes [2,3,5], and the high-salt portion of the biologically treated wastewater still requires further treatment. The traditional producers of pickled mustard leaves are small factories and individual farmers, who cannot afford a treatment facility or its maintenance. In addition to pickling water (PW), waste blades and stems are also difficult to treat and recycle through conventional methods, such as composting or disposal in combustion facilities and landfills, due to their high briny water contents [8]. Therefore, most pickling wastewater and solids are disposed of in nearby agricultural canals or small rivers. The disposals of saline wastewater and solids have created environmental pollution problems, including the salinization of water and deterioration of riverbanks downstream [7,9].

From an economic and sustainability viewpoint, it is necessary to develop a low-cost, low-maintenance method for treating the briny pickling wastewater that requires no specific physicochemical or microbial processes. Therefore, the objective of this study is to evaluate the potential of using pickling wastewater in fish culture. A preliminary experiment was conducted to determine the proper dilution ratios of PW mixed with seawater. The liquid contained in the waste blades and stems was also squeezed out and treated similarly to the PW to evaluate its potential utility in fish culture. In a second experiment, tilapia (*Oreochromis mossambicus*) was raised in the diluted water samples to evaluate its effects on fish culture. This study is the first propose the use of mustard leaf PWs in fish culture.

2. Materials and methods

2.1. Sample collection and treatment

Samples of PW, waste blades, and stems of pickled mustard (*B. juncea*) leaves were collected from a manufacturing area in Dapi, Yunlin County, southwestern Taiwan, in March 2009. To minimize changes after sampling, the PW was poured into jars and filled to the top to exclude air. The blades and stems were placed in plastic bags and sealed. The samples were kept in the dark at <10℃ and transported to the laboratory within 4 h.

Upon arrival at the laboratory, 50 g subsamples of the blades and stems was used to measure the water contents. The containing briny waters in the blades and stems were extracted by hand squeezing. Before squeezing, the stems and blades were separated into two subsamples to compare the effects of pretreatment. One pretreatment was to cut the blades and stems into pieces measuring approximately 1 cm^2; the other method was to leave the blades and stems untreated to simulate on site post-production conditions. This study expresses the squeezable water contents of the blades and stems as percentages (%) of the reduced weights of the samples.

2.2. Dilution with seawater

To determine the proper dilution ratios, the briny waters were diluted with seawater before their use in marine fish culture. The three briny water samples used in the dilution experiment included PW, water extracted from waste blades (BW), and water extracted from stems (SW). Each 500 mL of the PW, BW, and SW was diluted with seawater in a geometric sequence of 10. The dilution ratios ranged from 10^0 to 10^5. After being thoroughly mixed, characteristics of water samples were analyzed, including dissolved oxygen (DO), pH, salinity, suspended solids (SS), total phosphate (TP), total bacterial count (TBC), biochemical oxygen demand (BOD), nitrite–nitrogen (NIN), and ammonia–nitrogen (AN). All experiments were performed in triplicate. Table 1 lists the characteristics of the seawater used for dilution, which was collected at the seashore of Yunlin County near a marine aquaculture area.

2.3. Fish culture

The following experiment investigated the use of diluted and aerated waters for fish culture. The PW, BW, and SW samples were diluted by 10^3 with seawater and poured into rectangular ($200 \times 120 \times 75$ cm) fiberglass culture tanks to a fill depth of 40 cm (about 0.96 tons per tank). The control tank (Ct) was filled with seawater only. The bottom of each tank was covered with 5 cm of crushed coral sand for filtration. The waters were then aerated for 2 weeks before the fish culture experiment. During culture, the tanks were exposed to photoperiods of 16 h of light at an average intensity of 600 lux and 8 h of dark every day. Water temperatures were maintained at 27.5 ± 0.5℃. Each experiment was performed in triplicate. The experimental period was 1 month.

Table 1
Characteristics of seawater used for the dilution experiment

Item	Concentration
DO (mg L^{-1})	6.3
pH	8
Salinity (‰)	34
SS (mg L^{-1})	30
Total phosphorus (μg L^{-1})	<6
TP (μg L^{-1})	2
TBC (cfu mL^{-1})	58.7×10^4
BOD (mg L^{-1})	2
AN (μg L^{-1})	63
NIN (μg L^{-1})	<10

Juvenile tilapia, *O. mossambicus*, were obtained from the Mariculture Research Center of the Fisheries Research Institute, Taishi, Yunlin County, Taiwan. The juvenile tilapia had a mean initial weight of 44.9 ± 4.3 g at the start of the experiment. The stocking density was 25 fish per tank (about 1.2 g fish weight L^{-1}). Additional aeration was provided by compressed air through air stones during the experimental period. Fish were fed dry pelleted commercial feed (Chengon, Chiayi, Taiwan) with the following composition: 11% moisture, 23% crude protein, 3% crude fat, 6.5% crude fiber, and 12% ash. The daily amount of food remained at 2% of the fish weight throughout the experiment and was adjusted each week after all fish in each tank were weighed as a group. Fish were fed twice a day. Dead fish were not replaced, and the mortality rates in each group were recorded daily. The fish weight gain percentage (%), specific growth rate (SGR), and survival for each treatment were determined at the end of the experiment. The water from each tank was periodically sampled for water quality analysis. The analyzed characteristics of water quality included DO, pH, SS, AN, NIN, and nitrate-nitrogen (NAN).

2.4. Analytical procedures

The water content of the waste stems and blades was measured using a drying method. Ten grams of each sample was wiped dry, weighed, sliced, and dried at 105 ± 1℃. The water content of each sample was expressed as a percentage (%) of the reduced weight.

Salinity was measured by an S/Mill-E refractometer (Atago, Tokyo, Japan). DO was measured by a DO analyzer and a probe (Inolab Oxi 730, WTW, Weilheim, Germany) with salinity calibration. The pH levels of the water samples were measured by a pH meter and probe (PB-10, Sartorius, Goettingen, Germany). AN, NIN, NAN, TP, BOD, and SS were measured by the following methods [10,11]. AN was measured using the phenate method through reaction with phenol, forming indophenol in the presence of alkali and oxidizing agents. The AN level was detected at an absorbance of 640 nm. NIN was determined using a colorimetric method through coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylenediamine dihydrochloride. The NIN level was determined at an absorbance of 543 nm. The NAN analysis in this study used a cadmium-copper reduction method after treatment with NH_4Cl-EDTA and copperized cadmium. TP was measured using the molybdenum blue method after processing with ammonium persulfate and was determined at an absorbance of 880 nm. BOD was measured as the rate of oxygen uptake by microorganisms in the water sample at a temperature of 20 ± 1℃ over 5 days in the dark. SS was measured by filtering the samples through filters (GA-55, Advantec, Toyo Roshi, Tokyo, Japan) and drying them at 105 ± 1℃ until the weights were constant. The value of the TBC was enumerated by incubating 0.1 mL of a properly diluted water sample on plate count agar (Merck, Darmstadt, Germany) at 32℃ for 48 h. The number of bacteria was expressed in terms of colony forming units (cfus) mL^{-1}. The salinity of the agar was adjusted to 34‰ using NaCl.

The SGR of the fish in this study was calculated as $100 \times [(\mathrm{Ln}\,W_f - \mathrm{Ln}\,W_i)/t]$, where Ln is the natural log, W_f is final fish weight, W_i is the initial fish weight, and t is time in days.

2.5. Statistical analyses

This study uses an analysis of variance and Duncan's multiple-range test to determine significant differences between treatments in all experiments [12]. The significance level was set at 5%.

3. Results and discussion

3.1. Water content of pickled mustard solid waste

Pickled mustard leaf waste has a high water content. The average water contents of the waste blades and stems were 85 and 90%, respectively. Previous research reports that the water content of pickled mustard waste is approximately 81–82% [9], which is slight lower than the levels found in this study. This is perhaps due to discrepancies in product manipulation during the pickling process. In addition, the amount of water squeezed from the pickled blade and stem wastes increased after mincing pretreatment. Squeezing the blades and stems reduced their weights by 22 and 44%, respectively. In addition, the weights of the blades and stems decreased by 36 and 60% if minced before squeezing. This indicates that squeezing the minced pickled waste blades and stems can effectively reduce the briny water content.

3.2. Water quality after dilution treatment

The characteristics of the PW, BW, and SW significantly changed after being diluted with seawater (Table 2). For example, DO levels were low in all three briny waters. Decreased DO levels are common in PW due to fermentative microbial growth during the pickling processes [1]. This study shows that the DO levels of PW increased with increasing dilution ratios. Previous research suggests that the DO of an aquaculture pond should be $>5\,mg\,L^{-1}$ to maintain the activities of cultured and environmental organisms [13,14]. In the current study, a dilution ratio of 10 enabled the three briny waters to meet the minimal DO requirements for aquaculture pond environments. However, results show that a higher dilution ratio of 10^3–10^4 was necessary to reach the DO of the seawater ($6.3\,mg\,L^{-1}$) used for dilution.

All three briny water samples had low pH levels. The pH levels of both tissue-containing waters (3.7 for both BW and SW) were lower than the pH level of PW, at pH 4.3 (Table 2). A low pH level is a characteristic of PWs due to the proliferation and bioactivity of fermentative microbes [1,3]. Other pickling wastes and waters have similar pH levels; for example, the pH of pickling wastes is 3.96–3.97 [9] and 3.5–4.0 [1], 4.11–4.4 [15] in pickled mustard water, and 2.6–3 in pickling industrial wastewater [2]. The pH levels of the three brine water samples in this study increased with rising seawater dilution ratios. The optimum pH level suggested for the culturing of marine animals is usually pH 6.5–8.5 [13,16,17]. Results show that the pH level (6.7–7.7) of the water at a dilution ratio of 10^2 was already at a suitable pH level for aquaculture. However, the pH level increased to 7.8–7.9 at dilution ratios of 10^3 and 10^4, similar to the pH level of the seawater used for dilution.

All three water samples had high salinities. The salinity level of PW, at 180‰, was higher than the other two samples, which were both 160‰ (Table 2). This is perhaps because only part of the salt content of the PW entered the mustard tissues during the pickling process. In addition, the salinity level of PW in this study was higher than that reported in other studies; the salinities of pickled mustard wastewater are generally 80–100‰ [1]. The salinity level of pickled plum wastewater is approximately 150‰ [5], which is also lower than that of the PW in the present study. The salinities of the three briny waters were at the level of seawater after a dilution ratio of 10^3 (Table 2).

Similar to salinity, the SS levels of the three briny waters were also high. BW had the highest level of SS ($4{,}671\,mg\,L^{-1}$), followed by PW ($3{,}640\,mg\,L^{-1}$) and SW ($2{,}917\,mg\,L^{-1}$) (Table 2). The high SS in the BW might be due to the blade tissues being softer than the stems. Therefore, the squeezing process produced more tissue fragments in BW than in PW and SW. In addition, the SS contents of the three briny water samples in this study were much higher than those in other pickling wastewaters (40–$330\,mg\,L^{-1}$ [2,4]), but similar to the briny wastewater of fish canning factories (1.1–$2.1\,g\,L^{-1}$ [18]). High SS might affect cultured species by inhibiting the growth of phytoplankton and other aquatic organisms or clogging the gills of the culture species [13]. Optimal conditions for aquaculture require SS levels that do not to exceed $80\,mg\,L^{-1}$ [16]. Therefore, the SS levels of the three briny waters were within the usual ranges of aquaculture environments after dilution ratios reached $\geqslant 10^3$.

Based on 11–$13\,\mu g\,L^{-1}$ at dilution ratios of 10^2 (Table 2), the original TP concentrations in the three raw briny waters were approximately 930–$1{,}200\,\mu g\,L^{-1}$. The concentrations of TP in waters of this study were lower than those reported for raw pickling wastewater (22–$25\,mg\,L^{-1}$ [2]). The TP levels in most aquaculture ponds are 170–$400\,\mu g\,L^{-1}$ and seldom exceed $1{,}000\,\mu g\,L^{-1}$ [16]. This indicates that a dilution ratio of 10^2 (11–$13\,\mu g\,L^{-1}$) or higher ($<6\,\mu g\,L^{-1}$) can produce TP levels that are similar to the usual concentrations in aquaculture environments.

The three briny water samples had high TBCs. Although they decreased with dilution, the TBCs of the three briny waters were still about 6×10^4–$29\times10^4\,cfu\,mL^{-1}$ at dilution ratios of 10^4 (Table 2). The process of pickling mustard greens includes the proliferation of naturally occurring fermentative lactic acid bacteria, which carry out the fermentation [1]. This may be why TBCs were high in the three diluted briny waters. Another possible reason was the high TBC ($5.87\times10^5\,cfu\,mL^{-1}$) in the seawater used in this dilution experiment, which was sampled from the seashore near marine aquaculture areas. While there are no suggested criteria for TBC in aquaculture, previous research reports TBCs of approximately 1.8×10^2–6.0×10^4 [19] and 1.4×10^3–$8.6\times10^3\,cfu\,mL^{-1}$ [20] in brackish tilapia ponds. Both of these TBCs were both lower than the TBC in the water sample with the highest dilution ratio (10^4) in this study.

Table 2
Water quality of the three briny water samples after dilution with different ratios of seawater. The briny water samples included PW and waters contained within the waste pickled blades (BW) and SW. This table represents each water quality parameter using the average concentration and corresponding standard deviation (in parentheses) for $n = 3$

Water	Water	Dilution ratio				
Quality item	Sample	$\times 10^0$	$\times 10^1$	$\times 10^2$	$\times 10^3$	$\times 10^4$
DO ($mg\,L^{-1}$)	PW	–*	6.2	5.8	6.2	6.0
		–	(0.1)	(0.0)	(0.1)	(0.1)
	BW	–	5.3	5.5	5.5	5.8
		–	(0.0)	(0.0)	(0.0)	(0.0)
	SW	–	5.4	5.7	5.9	6.1
		–	(0.1)	(0.1)	(0.1)	(0.1)
pH	PW	4.3	6.1	7.7	7.9	7.9
		(0.0)	(0.0)	(0.0)	(0.0)	(0.1)
	BW	3.7	4.3	6.8	7.9	7.9
		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
	SW	3.7	4.1	6.7	7.8	7.8
		(0.0)	(0.0)	(0.0)	(0.0)	(0.1)
Salinity (‰)	PW	180	49	35	34	34
		(0)	(0)	(0)	(0)	(0)
	BW	160	47	36	34	34
		(0)	(0)	(0)	(0)	(0)
	SW	160	47	35	34	34
		(0)	(0)	(0)	(0)	(0)
SS ($mg\,L^{-1}$)	PW	3,640	371	107	45	30
		(71)	(19)	(7)	(3)	(9)
	BW	4,671	328	111	43	30
		(150)	(23)	(7)	(4)	(6)
	SW	2,917	305	118	48	31
		(189)	(20)	(14)	(7)	(1)
Total phosphorus ($\mu g\,L^{-1}$)	PW	–	–	11	<6	<6
		–	–	(0)		
	BW	–	–	13	<6	<6
		–	–	(0)		
	SW	–	–	12	<6	<6
		–	–	(1)		
Total bacteria (cfu $\times 10^4\,mL^{-1}$)	PW	–	–	7,600	350	29
		–	–	(360)	(35)	(7)
	BW	–	–	2,000	230	13
		–	–	(400)	(21)	(3)
	SW	–	–	340	65	6
		–	–	(12)	(2)	(2)
BOD ($mg\,L^{-1}$)	PW	–	–	–	6	3
		–	–	–	(0)	(0)
	BW	–	–	–	5	5
		–	–	–	(0)	(0)
	SW	–	–	–	5	5
		–	–	–	(0)	(0)

(*Continued*)

Table 2
(*Continued*)

Water Quality item	Water Sample	Dilution ratio $\times 10^0$	$\times 10^1$	$\times 10^2$	$\times 10^3$	$\times 10^4$
NIN ($\mu g\,L^{-1}$)	PW	–	11	<10	<10	<10
		–	(0.4)			
	BW	–	30	<10	<10	<10
		–	(2)			
	SW	–	12	<10	<10	<10
		–	(1)			
AN ($\mu g\,L^{-1}$)	PW	–	–	77	79	61
		–	–	(13)	(7)	(3)
	BW	–	–	45	52	66
		–	–	(4)	(6)	(4)
	SW	–	–	50	61	66
		–	–	(3)	(2)	(13)

*Undetectable data due to interferences from high salinities or the dark-brown color of the water samples.

BOD is a common parameter for evaluating the oxygen demand of water. The BOD levels of the three water samples in this study were <5–6 $mg\,L^{-1}$ at a dilution ratio of 10^3 (Table 2). BOD is also a key factor in aquaculture systems, because if it is too high, aquatic animals may be stressed or killed by the resulting low DO concentrations [13]. The BOD of aquaculture pond water should not exceed 30 $mg\,L^{-1}$ [14]. Therefore, a dilution ratio of 10^3 or higher was necessary for the three briny waters to meet this criterion.

NIN can be toxic to aquatic organisms if it accumulates in aquatic systems [16]. This study shows that the NIN levels in the three diluted briny waters were low. The NIN levels of the three diluted water samples all decreased to <10 $\mu g\,L^{-1}$ when the dilution ratio was increased to 10^2 (Table 2). Pickling wastewater typically has very low levels of oxidized nitrogen, including nitrite and nitrate (<1 $mg\,L^{-1}$ [4]). This can be attributed to the inhibition of nitrification and denitrification activities at high salinities [4,21]. Adequate concentrations of nitrite are 1.3–10.6 $mg\,L^{-1}$ in aquaculture pond water [13], which is much higher than the levels in the diluted water samples in this study.

The AN levels in the three diluted briny water samples were low and inconsistent with increasing dilution ratios. The AN concentrations in the three diluted samples were approximately 45–79 $\mu g\,L^{-1}$ at dilution ratios of 10^2–10^4 (Table 2). This might have been a result of the background concentrations of AN reaching 63 $\mu g\,L^{-1}$ in the seawater used for dilution. Ammonia is acutely toxic to aquatic life. Its degree of toxicity depends on its chemical state, environmental conditions, and the species and life stage of the cultured organisms [22]. The AN in culture water should generally be kept at <0.2 $mg\,L^{-1}$ [13]. Therefore, a dilution ratio of $\geqslant 10^2$ allowed the three briny water samples to meet this aquaculture criterion.

3.3. Fish culture

Table 3 summarizes the mean values of weight gain, SGR, and survival in fish culture. At end of the culture period, tilapia juveniles in the PW, BW, SW, and control samples had weight gains of 20.5, 22.9, 22.1, and 15.3%, respectively (Table 3). In addition, the SGRs were 0.66, 0.74, 0.71, and 0.51% d^{-1} for the PW, BW, SW, and control samples, respectively. There were no significant differences in the weight gain or SGRs of tilapia between any of the treatments and the control. This indicates that all the three pickling briny waters should allow viable growth of tilapia after being diluted with seawater at $\geqslant 10^3$.

The pH, DO, and SS levels remained at approximately 7.7–8.1, 7.5–8.0, and 5–10 $mg\,L^{-1}$, respectively. Furthermore, decreases in AN and NIN with a concurrent increase in NAN in waters during the experiment indicated continued nitrification in the culture tanks (Fig. 1). On the other hand, tilapia survival rates were significantly lower in PW (92%) than in BW or SW (both 100%). The lower survival rates in PW might be attributed to AN concentration fluctuations during days 14–21 (Fig. 1). AN is very toxic to fish and should be kept at <0.2 $mg\,L^{-1}$ [13]. Although lower than the suggested concentration, the fluctuation of AN in the PW tanks can still stress the fish and adversely affect their survival. The nitrate accumulated gradually to the end of study with concentrations between 400 and 600 $\mu g\,L^{-1}$. Nitrate was

Table 3
Weight gain, SGRs, and survival rates of tilapia cultured in the diluted PW, BW, and SW for four weeks. Data are presented as the mean and corresponding standard error, $n = 25$ fish

Water	PW	BW	SW	Control
Weight gain (%)	20.5 ± 4.0^a	22.9 ± 5.3^a	22.1 ± 4.2^a	15.3 ± 5.0^a
SGR (% day^{-1})	0.66 ± 0.14^a	0.74 ± 0.19^a	0.71 ± 0.15^a	0.51 ± 0.17^a
Survival rate (%)	92^b	100^a	100^a	100^a

^{a,b}Values with different superscripts within a row exists significant differences ($p < 0.05$).

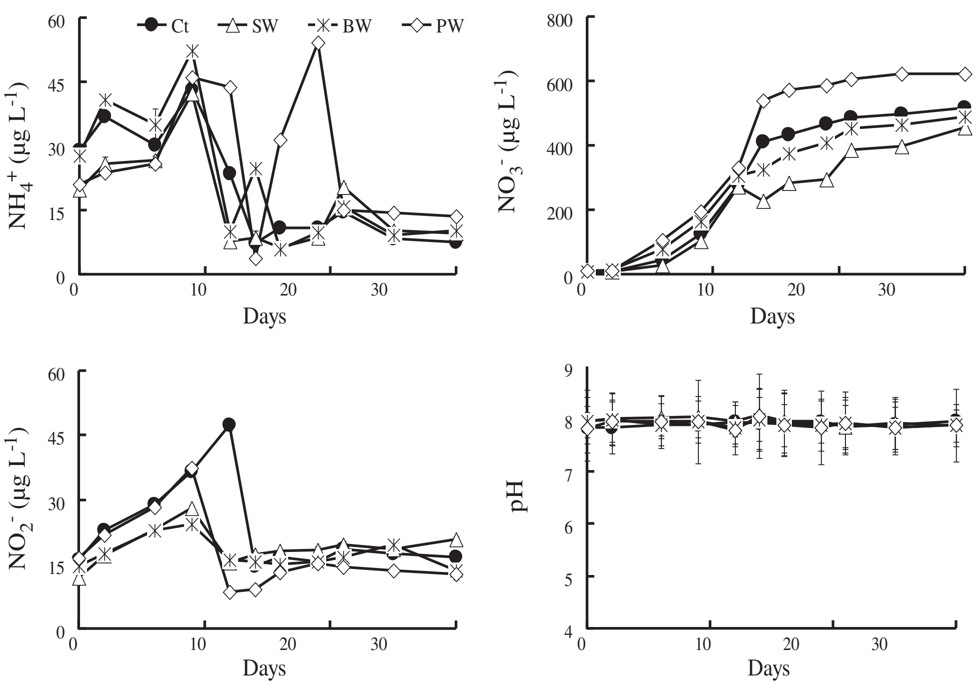

Fig. 1. Concentration changes of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and pH in water samples containing juvenile tilapia, *O. mossambicus*. Tank waters included seawater-diluted PW and waters squeezed from BW and SW. The Ct contained seawater only.

suggested having no significant toxicity to fish below 20 mg NO_3–N L^{-1} [23], which is much higher than the nitrate concentration in the present study, and, therefore, no negative effect to the fish was considered. In conclusion, the three treated PWs in this study are not harmful to fish and can be used in marine fish culture. This is an alternative method for utilizing pickling wastewater and reducing the briny water content of pickled mustard leaf wastes.

4. Conclusions

The briny water produced by pickling mustard leaves is typically treated as industrial wastewater, while the unusable blades and stems are briny wastes. This study utilizes the PW and the water extracted from waste blades and stems of pickled mustard leaves to raise marine tilapia fish. Result indicates that after a dilution with seawater to 10^{-3}–10^{-4} and aeration, these briny waters were suitable for use in marine fish culture. Most of the marine fish ponds were established nearby and/or having canals connecting the sea and easily to get plenty of seawater for culturing the marine fish. Therefore, the dilution and aeration of the pickling wastewater can be achieved with the current facilities in the aquaculture farm. This is an alternative method for re-using the PW and reducing the briny water content of pickled mustard leaf wastes. The treated PW can be re-used in marine fish culture instead of being treated as industrial wastewater.

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