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Evaluation of a Small Scale UV-treated Recirculating Depuration System for Oysters (*Crassostrea iredalei*)

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Abstract Oysters are filter-feeding organisms that tend to concentrate any suspended materials in its surrounding water including pathogenic bacteria. Since most oysters are eaten as raw or slightly cooked, they can act as vectors for pathogenic microorganisms and thus impose health risks to consumers. Depuration is one of the methods to reduce pathogenic bacteria in oysters to make it safe for sale and consumption. This study was designed to evaluate the effectiveness of the small scale UV- treated recirculating depuration system manipulating different parameters such as water flow rate and tank density. It aims to determine the effect of this UV-treated recirculating depuration system in reducing pathogenic bacteria in oysters such as *E. coli, Salmonella, Vibrio cholera* and *Vibrio parahaemolyticus,* and with the survival rate and meat yield of oysters. The experimental results showed that the depuration system was effective in reducing *E. coli* at different water flow rate (15L/min, 10L/min and 5L/min), in all density level (2 oysters/L, 4 oysters/L and 6 oysters/L). However, for *Vibrio parahaemolyticus* and *Vibrio cholera* reduction, only water flow rate of 15L/min at density level of 2 oysters/L and 4 oysters/L revealed to be effective. The survival of the oysters was high in treatments with a density of 2 oysters/ L. The meat yield revealed to have no significant difference *(P*<0.01) between treatments with water flow rates. In general, treatment with water flow rate of 15 L/min in combination with 2 oysters/L density showed most promise results on all analysis.

Keywords: *oyster, recirculating, depuration system, E.coli, Vibrio, flow rate, density*

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1. Introduction

Oysters (*Crassostrea iredalei*) are soft-bodied bivalve mollusk, usually found in the marine or estuarine environment [1]. They can grow in all tropical seas and are considered to be a valuable food item. They constitute a rich source of essential macro and micronutrients in providing a balanced diet [1]. The edible oysters are very popular food in the South East Asian countries, Europe, Australia, and USA [1]. Oysters are usually consumed raw. This leads to the potential risks and transmission of pathogenic microorganisms from contaminated areas [2].

The consumption of contaminated bivalve shellfish is associated with a number of human diseases, particularly when oysters are ingested as raw [3].Since oysters are filter feeders, they tend to absorb and concentrate pathogenic microorganisms; particularly when the shellfish originated from contaminated areas [2].Seafood is the main source of animal protein by more than a billion people globally, and contaminated seafood is a frequent etiology of diseases contracted from the ocean, including both pathogenic and chemical contamination [4]. Coliform bacteria and *Escherichia coli* are common indicators of microbiological infection of shellfish and monitoring shellfish contamination. However, monitoring shellfish bacterial contamination does not always detect viral contamination [5]. Health problems due to contaminated bivalve shellfish have been described and viral contamination has been linked to numerous cases of gastroenteritis, as well as outbreaks of various illnesses [4].

The microbiological quality of bivalves is closely related to the aquatic habitat that varies with factors such as environmental conditions and bacterial load of the water [6]. Contamination of mollusks with human pathogenic bacteria is linked to wastewater, and which are naturally present in coastal environments [7]. Contamination of bivalve shellfish occurs mainly because they are suspension feeders that selectively filter small particles of phytoplankton, zooplankton, viruses, bacteria and inorganic matter from the surrounding water [8,9]. The Food and Drug Administration [10] have required the shellfish industry to use fecal coliforms as indicators of contamination in harvesting waters and oysters. If the *E. coli* is detected above the most probable number (MPN) of 230/100 g of oyster, then the shellfish is subject for purification. Further, European Union (EU) regulations for mollusks, in addition to the absence of *Vibrio sp.* and *Salmonella sp.,* a maximum tolerable cell concentration is required both for fecal coliforms (300 MPN/100 g meat) and *Escherichia coli* (230 MPN/100g meat) [11]. One strategy developed for the management of this risk is the depuration of the bivalves.

The risk of illness associated with the consumption of bivalves can be reduced by growing or farming of shellfish in

areas with low levels of microbial contaminants. The risk may be further reduced by treating the shellfish such as depuration [12]. Depuration is a commercial practice where the harvested shellfish are placed in tanks filled with clean seawater for several hours to allow the shellfish to filter and their purge contaminants [2,13]. This method reduces the levels of microorganisms present in mollusc tissue, thus decreasing the potential for infections that are associated with shellfish ingestion. Depuration of shellfish is usually employed to reduce the bacterial load present and likewise decrease the potential for infections associated with shellfish consumption. There are two types of depuration system being used: flow through and recirculating. Flow through system uses natural seawater to continuously flow on the tanks, making it susceptible to fluctuations in the microbial community composition [1]. Recirculating system requires artificial seawater to be constantly cycled through after sterilization [15].

The use of ultraviolet (UV) for disinfecting water is commonly used in a depuration system. Depuration using UV light is usually effective provided that the water flow rate is adequate to its capacity [15]. UV disinfection technology can be readily applied, is low maintenance, results in significant viability reductions of all waterborne pathogens, and produces no hazardous by-products [16,17]. UV radiation eliminates enteric bacteria, viruses, bacterial and protozoan spores in the water without the production of toxic by-products or other chemical residues [13]. Also, depuration effectively inactivates *Salmonella typhimurium* from oysters when UV radiation and chlorine treatment are employed for at least 12 h [18].

The technology of oyster depuration has been well studied in different countries of temperate regions [1,19,20], and is reviewed by Oliveira et al. [21]. However, there is very limited scientific information regarding oyster depuration in the tropical regions using a small scale UV-treated recirculating depuration system. In the Philippines, there are no reports on depuration using recirculating system in combination with UV as disinfection for the purification of oysters [1].

The aim of this study was to evaluate the effectiveness of the small-scale UV-treated recirculating depuration system to reduce bacterial load in oysters by manipulating water flow rate and oyster density.

2. Materials and Methods

Approximately 80 kilos of oyster (*Crassostrea iredalei*) samples measuring 50-60 mm shell length, 40-30 mm shell width, 5-10 mm shell thickness, and weighing 40-50g were collected in the coastal part of Dumangas, Iloilo, Philippines (10°47'52.8"N 122°40'25.9"E). The selection of the sampling site was based on the data of oyster production of Region VI in the Philippines, where the area was identified as one of the major producers [22]. Ease of transportation was also considered. The production in the area was sufficient enough to provide samples for the experiment.

2.1. Collection of Oyster Samples

Oysters were transported from the site to the UPV laboratory in Miagao, Iloilo, Philippines, within 2 hours. They were carried in a perforated styrophore box, covered with damp cloth to keep them moist. Oysters were cleaned immediately after arrival by gently brushing to remove mud, sand, barnacles and other unwanted particles. Samples were carefully inspected; dead or damaged specimens were eliminated. Only live and healthy oysters were subjected for the depuration process. The depuration system was disinfected by UV for 12 h prior to the placement of oysters in tanks and depuration process.

2.2. Recirculating Depuration System

The depuration tank is a closed system that re-circulates seawater. The depuration system consisted of nine 66x43x28 cm (50 L) depuration tanks with perforated plastic trays (2 trays per tank) for the placement of oysters (Figure 1). The water from the storage water tank (155 L) is recirculated by a submersible pump (1/2 horse power) at an adjusted rate of 5400 L/h. The sterilizing system consisted of one 55 Watts UV (24 gallon/min.) unit and a water filter with 10 µm filter cartridge. The water flow rate in every tank was adjusted using a control valve. The depuration tank has a water level control outlet which enables the water to flow out of the tank without overflowing. From the outflow, water goes back to the storage water tank passing through a cloth filter to remove dirt from the depuration tanks.

2.3. Depuration Experiment

Oysters were placed in the plastic trays, raised at least 25 mm off the bottom of the tank to restrict recontamination with feces and accumulated dirt. Artificial seawater was used in the experiment in order to control the salinity of the water. The salinity and temperature in the depuration tanks were remained constant. The salinity of the water was maintained at 25 ppt (parts per thousand) and temperature was at ambient state i.e. 28-30 °C. The water flow rate was adjusted to 5, 10 and 15 L/min. while the oyster density was 2 oysters/L, 4 oysters/L and 6 oysters/L. Tank with static (no water flow) artificial seawater was maintained as the control. Water parameters such salinity, temperature and dissolved oxygen was monitored at 0, 24, and 48 h. The depuration assays were performed for a total of 48 h.

2.4. Experimental Design

The Experimental design employed in this experiment is 3 x 4factorial with four levels of treatments of water flow rate and three levels of oyster density. Treatment combination in the experiment is executed three times on every level of water flow rate (F) and oyster density (D), as shown in Table 1.

Table 1. Factorial combination of treatments of water flow rate and oyster density

Factors		Flow rate (F)			
		F1	F2	F3	F4
		Static	5L/min	10L/min	15 L/min
Density (D)	D1 2oysters/L	F1D1	F2D1	F3D1	F4D1
	D2 4oysters/L	F1D2	F2D2	F3D2	F4D2
	D3 6oysters/L	F1D3	F2D3	F3D3	F4D3

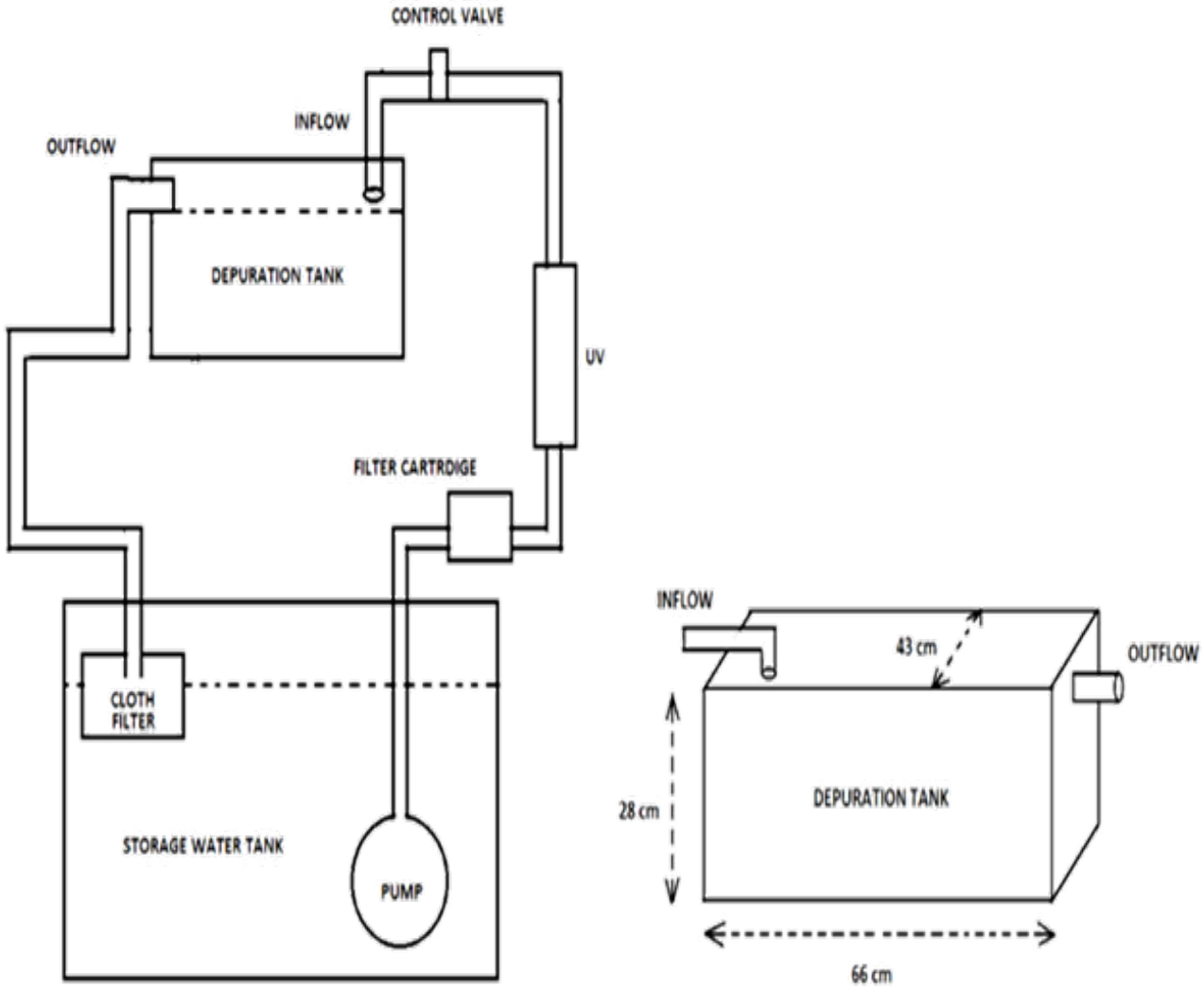

Figure 1. Diagram o the small-scale recirculating depuration system for oysters

2.5. Enumeration of *E. coli* (MPN Method)

E. coli was determined using the conventional five-tube, 3-dilution MPN method [40]. One hundred grams oyster meat sample was homogenized in 100 ml of 0.1% phosphate buffer. Dilution tubes (up to 10^3) were prepared and 2 ml of each dilution was inoculated into each tube of lactose broth. Each tube contained inverted Durham tubes. Tubes were incubated at 35°C for 24-48 hrs. All tubes that showed turbidity and gas production were inoculated to *E. coli* broth and incubated at 44.5°C in water bath for 24 hrs.

A loopful of sample from positive *E. coli* broth was inoculated in tryptone and incubated for 24 h at 35°C. *E. coli* was confirmed positive through indole production in tryptone by adding Kovac's reagent [24,25]. Quantification was determined using the MPN table and *E. coli* was reported as MPN /100g sample. Analysis was done before and after the depuration process.

2.6. *Vibrio parahaemolyticus* (MPN Method)

Vibrio parahaemolyticus was determined using MPN method. A 25-gram homogenized sample was mixed in 225 ml 0.1% alkaline peptone water (APW) supplemented with 3% NaCl. Decimal dilutions were prepared (A, 10^2, 10^3) and 1 ml of each dilution was transferred to three tubes containing 10 ml 0.1% APW with 3% NaCl. They were incubated at 35°C for 24 hrs. After incubation, they were streaked on thiosulfate-citrate bile salts-sucrose agar (TCBS) plates and were incubated for 24 hours at 35°C. Blue-green colonies were determined as *Vibrio parahaemolyticus* and were subjected to biochemical screening [23]. Quantification was determined using the MPN table and was reported as MPN /g sample.

2.7. *Vibrio cholerae* Detection

Twenty five grams of homogenized sample was mixed in 225 ml of 0.1% alkaline peptone water (APW) added with 3% NaCl. Serial dilution was performed (10^2, 10^3) in 9ml APW tubes. They were incubated for 6-8h and 16-24 hat 37°C and 42°C. A loopful from each tube was streaked into two plates of pre-poured thiosulfate-citrate bile salts-sucrose agar (TCBS) and was incubated for 24 h. Yellow typical colonies were determined as *Vibrio cholera* and were subjected to biochemical screening [23].

2.8. Survival and Meat Yield of Oysters

Live oysters were counted after the depuration experiment to calculate the percentage survival rate. The meat yield was determined by shucking the oysters and weighing the meat using an electronic weighing balance.

Percentage meat yield was calculated using the formula below [26,27]:

$$\text{MY}\ (\%) : \left[\text{wet meat weight}\ (\text{g}) / \text{total weight}\ (\text{g})\right] \text{X}100.$$

2.9. Physico-chemical Parameters

Water parameters such as salinity, temperature and dissolved oxygen (DO) were monitored at 0, 24 and 48 h using refractometer, thermometer and DO meter, respectively. Monitoring of water parameters should be undertaken during depuration, at the beginning, in the middle and at the end [1].

2.10. Statistical Analysis

Statistics were performed using SPSS Version 20 (SPSS Inc., USA). A univariate ANOVA was used to determine the effects of water flow rate and oyster density on the bacterial count. A Duncan post-hoc test was run to determine which factors were significant. All tests were set at a significance level of $p<0.01$.

3. Results and Discussion

3.1. Physicochemical Parameters of the Recirculation Water in the Depuration System

The physicochemical parameters of the recirculated artificial seawater in the depuration system are shown in Table 2. The salinity and temperature of the water in the sampling site were determined to be 25 ppt and 30°C respectively. The salinity of the water in the depuration system was kept at 25 ppt and the temperature varies from 28-30°C. Reports have demonstrated that the optimal temperature and salinity of depuration process water are related to the ambient environmental conditions at the harvest site [28]. In this study, the temperature and salinity of the water in the sampling site and in the depuration system were almost similar. The Food and Agriculture Organization [1] had recommended a salinity of 25 ppt for oysters (species not identified) in a depuration process. In the Philippines, a minimum salinity of 17.5 ppt for *Crassostrea iredalei* during depuration process is specified [29]. For dissolved oxygen requirement, above 5 mg/L is recommended in a depuration system [1].

3.2. Initial Count of Bacteria in Oysters

The initial count of bacteria in oysters was determined prior to every depuration experiment (Table 3). Based on the data, the initial count of bacteria in oysters were beyond the standard microbiological limit. It was observed that in the third run, higher levels of *E. coli* and *Vibrio parahaemolyticus* were recorded compared to the previous runs. This might be due to the different sampling time in every run. Run 1 and 2 were conducted on the month of March, while run 3 was conducted on the month of April. Several studies proved that the microbial load of oysters varies over time [30], which is influenced by weather, distribution, and rate of pollution in the harvesting area [7,31,32].

3.3. Reduction of *E. coli* Count in Oysters

The mean MPN count and percentage reduction of *E. coli* in oysters after the depuration process are shown in Table 4. Based on the results, all treatments with water flow rates (L/min) of 5, 10 and 15 (F2, F3, F4) in all density levels have reduced *E. coli* count in oysters to an acceptable microbiological limit of 230 MPN /100g. Treatment with water flow rate of 15 L/min in combination with 2 oysters/L density (F4D1) has the lowest count of 12 MPN /100g. Treatments with no water flow rate or static (F1) in all density levels retained high count after the depuration process.

Treatments with water flow rate of 15 L/min in combination with 2 oysters/ L density (F4D1) has the highest *E. coli* reduction of 96.3%. But has no significant difference ($p<0.01$) to treatments with lower water flow rate of 10 L/min at different oyster densities (F3D1, F3D2 and F3D3). Treatments with no water flow rate or static (F1) in all density levels have the lowest reduction ranging from 12.3 to 25.9% only. This result suggests that *E. coli* can be reduced in any water flow rates and oyster density levels. Using static method of depuration is not effective in reducing high levels of *E. coli* in oysters.

Table 2. Physicochemical parameters of recirculated water in the depuration tanks in all treatments

Treatment	Salinity (ppt)	Temperature (°C)	Dissolved oxygen (mg/L)
F1D1	25	28	5.0
F1D2	25	28	4.5
F1D3	25	28	4.4
F2D1	25	30	6.0
F2D2	25	30	5.6
F2D3	25	30	5.5
F3D1	25	30	6.3
F3D2	25	30	5.6
F3D3	25	30	5.5
F4D1	25	30	6.3
F4D2	25	30	5.7
F4D3	25	30	5.5

Table 3. Initial count of pathogenic indicators in oysters prior to depuration process.

Pathogenic bacteria	Initial count			Standard limit (USFDA, etc.)
	R1	R2	R3	
E. coli	280	220	350	<230 MPN/100g
V. parahaemolyticus	240	240	290	100 MPN/g
Vibrio cholera	Positive	Positive	Positive	Negative
Salmonella	Negative	Negative	Negative	Negative

Legend: (R1) = First run: March 6, 2017, (R2) = Second run: March 20, 2017, (R3) = Third run: April 3, 2017.

Table 4. Mean MPN count and percentage reduction of *E. coli* in oysters after depuration process in all treatments

Treatment	*E. coli* count MPN/100g				% reduction (Mean ± SD)*
	R1	R2	R3	Mean	
F1D1	220	140	280	213	25.9 ± 9.1 [a]
F1D2	240	170	280	230	19.0 ± 4.3 [ab]
F1D3	280	170	350	267	12.3 ± 11.5 [b]
F2D1	23	17	90	43	87.1 ± 8.6 [cd]
F2D2	30	21	80	44	84.7 ± 9.0 [cd]
F2D3	40	27	90	52	82.6 ± 7.2 [c]
F3D1	17	4	50	24	92.9 ± 6.5 [de]
F3D2	21	7	70	33	90.4 ± 9.2 [cde]
F3D3	14	6	70	30	89.8 ± 8.7 [cde]
F4D1	7	2	27	12	96.3 ± 3.6 [e]
F4D2	9	2	30	14	95.8 ± 3.9 [e]
F4D3	9	2	40	17	94.8 ± 5.5 [e]

The results of this study proved the effectiveness of the small scale UV-treated recirculating depuration system in reducing *E. coli* in oysters *Crassostrea iredalei.* Similar results were reported where 79% reduction of *E. coli* was observed using a UV-treated recirculating depuration set-up for 24 h with a water flow rate of 5.5 L/m and oyster density of 2 oysters/L [33]. In this study, treatments with water flow rate of 5 L/min in combination with 2 oysters/L density (F2D1) reduced *E. coli* by 87.1%. Likewise, reduction of naturally occurring *E. coli* counts in the Japanese oyster *C. gigas,* to less than the detection limit (30 *E. coli* MPN/100 g) after depuration with UV-treated seawater for 24 h at a rate of 10 L/min were reported [34]. In this study, treatments with flow rate of 10 L/min (F3) have reduced *E coli* count to 24-33 MPN/100g.

Furthermore, significant reductions of *E. coli* counts reaching undetected levels using UV treated recirculating depuration set-up for 48 hours with a flow rate of 2.9 L/min and a density of 2 oysters/L were also reported [35]. Also, reduction of *E. coli* to less than 230 MPN/100g for 48 h using UV treated seawater at a density of 2 oysters/L was studied [36]. Also, reduction of *E. coli* to less than 230 MPN/100g for 48 h using UV treated seawater at a density of 2 oysters/L was studied [36].

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3.4. Reduction of *Vibrio* in Oysters

Table 5 shows the mean MPN count and percentage reduction of *Vibrio parahaemolyticus* in oysters after the depuration process. Results indicated that only treatments with water flow rate of 15 L/min in combination with densities of 2 oysters/L and 4 oysters/L (F4D1 and F4D2) have reduced *V. parahaemolyticus* in oysters below the microbiological standard limit of 100 MPN/g. In terms of percentage reduction, treatment with water flow rate of 15 L/min in combination with 2 oysters/L density (F4D1) has the highest reduction of 73.6%. But shows no significant difference ($P<0.01$) to F4D2 (15 L/min: 2 oysters/L) and F3D1 (10 L/min: 2 oysters/L) treatments with a reduction of 66% and 52.9%, respectively.

Detection of *Vibrio cholera* in oysters after depuration process is shown in Table 6. Based on the data, only treatments with water flow rate of 15 L/min in combination with 2 oysters/L and 4 oysters/L densities (F4D1 and F4D2) had eliminated the bacteria in oysters. Other treatments including the static (control) retained its presence after the 48 hours depuration process.

The results of this study concur with other investigations which reported the persistence of *Vibrio* species in shellfish after depuration in UV-treated recirculating system. Depuration is a very effective process for the elimination of faecal bacteria, such as *E. coli,* but is less effective for naturally occurring *Vibrio* species [2].Low temperature depuration is recommended by several reports to reduce *Vibrio* in shellfishes [2,37,38]. Several studies reported that depuration process at ambient temperatures of above 20°C is not effective for eliminating *Vibrio spp*. in shellfish [39,40,41]. The ineffectiveness of depuration at ambient temperatures for reducing levels of *Vibrio* in oysters might be due to multiplication of *Vibrio* cells in oyster tissues when the water temperature increased to 26°C or higher [14]. The optimal growth temperature for *V. parahaemolyticus* is between 30 and 35°C with an upper growth limit of 45.3°C [14]. In this study, the temperature of the water in the depuration system was at ambient state (28-30°C) during the entire experiment. Persistence of the bacteria may be due to the ambient temperature of the water in the depuration system.

Significant change in levels of *V. parahaemolyticus* in oysters *Crassostrea gigas* depurated in UV-treated artificial seawater at ambient temperature (25°C) for up to 24 h were reported [42]. However, depuration at low temperature of 15°C has been reported capable of reducing *V. parahaemolyticus* in the *Crassostrea virginica* by 2.1 and 2.9 log MPN/g, after 48 hours depuration in UV-treated depuration system [43]. Depuration with refrigerated seawater at 5°C reduced *V. parahaemolyticus* populations by >3.0 log MPN/g in the Pacific oysters *Crassostrea gigas* without significant fatality of the oysterswere demonstrated [38]. Furthermore, a reduction of 10 MPN/g in the count of *V. parahaemolyticus* and *Vibrio cholerae* in oysters after 44 h of depuration at 16 to 18°C was reported [2].

Table 5. Mean MPN count and percentage reduction of *Vibrioparahaemolyticus* in oysters after depuration process in all treatments

Treatment	*Vibrio parahaemolyticus* MPN/g				% reduction (Mean ± SD)*
	R1	R2	R3	Mean	
F1D1	210	150	210	190	25.9 ± 12.6 ^{abc}
F1D2	210	150	290	217	22.4 ± 13.3 ^{ab}
F1D3	240	210	240	230	9.9 ± 8.9 ^{a}
F2D1	150	93	150	131	49.0 ± 11.9 ^{cde}
F2D2	160	120	150	143	43.9 ± 9.2 ^{bcde}
F2D3	210	160	240	203	21.0 ± 10.9 ^{ab}
F3D1	120	120	150	120	52.9 ± 4.9 ^{def}
F3D2	120	120	120	130	49.4 ± 0.9 ^{cde}
F3D3	150	210	160	173	31.6 ± 16.9 ^{abcd}
F4D1	64	64	75	68	73.6 ± 0.5 ^{f}
F4D2	93	75	93	87	66.0 ± 4.1 ^{ef}
F4D3	160	150	210	173	32.8 ± 4.9 ^{abcd}

* Non-identical superscript letters indicate a significant difference at P < 0.01.

Table 6. *Vibrio cholerae* in oysters after depuration process in all treatments

TREATMENT	*Vibrio cholera*			
	R1	R2	R3	Mean
F1D1	+	+	+	+
F1D2	+	+	+	+
F1D3	+	+	+	+
F2D1	+	+	-	+
F2D2	+	+	-	+
F2D3	+	+	+	+
F3D1	+	-	+	+
F3D2	+	-	+	+
F3D3	+	+	+	+
F4D1	-	-	-	-
F4D2	-	-	+	-
F4D3	+	-	+	+

Legend: (+)= positive, (-)= negative, (R1)= first run, (R2)= second run, (R3)= third run.

3.5. Survival Rate of Oysters after Depuration Process

Table 7 shows the percentage survival of oysters after the 48 hours depuration process. Based on the results, treatments with water flow rate of 15 L/min in combination with 2 oysters/L density (F4D1) has the highest survival rate of 85.1%. But shows no significant difference (P<0.01) to F2D1 (5 L/min: 2 oysters/L) and F3D1 (10 L/min: 2 oysters/L) treatments with a survival rate of 82.9%. It was observed that all treatments having a high density of 6 oysters/L (D3) have low survival rate ranging from 50.41 to 53.3%. It may be due to the low dissolved oxygen level in treatments with higher densities (Table 2) compared to treatments with lower densities.

3.6. Meat yield of Oysters after the Depuration Process

The meat yield of the oysters was determined after 48 h of depuration (Table 8). Based on the results, treatments with no water flow rate or static at different oyster densities (F1D1, F1D2 and F1D3) have the highest meat yield ranging from 18.7 to 18.9% with a percentage reduction of 0.75 to 4.9. Treatments with water flow rates (F2, F3 and F4) in all density levels have lower meat yield ranging from 14.5 to 16.4% only with a percentage reduction of 13.8 to 21.7. The result suggests that the meat yield was influenced by the water flow rate. However, there are no reports regarding the effect of flow rate on the meat yield of oysters during depuration. The decrease of meat yield in oysters may be due to the purging of contaminants including bacteria and other organic matter into the water, and are being washed away from the tanks. While oysters in static condition purge contaminants into the water and remains in the tank throughout the depuration process. Thus, there is a possibility that the contaminants where again filtered and accumulated in the oysters.

Table 7. Average percentage survival of oysters after depuration process in all treatments

TREATMENT	% Survival rate (Mean ± SD)*
F1D1	75.9 ± 2.0 ^{a}
F1D2	61.5 ± 1.2 ^{b}
F1D3	52.4 ± 2.3 ^{c}
F2D1	82.9 ± 1.3 ^{d}
F2D2	71.9 ± 2.1 ^{a}
F2D3	52.0 ± 5.5 ^{c}
F3D1	82.9 ± 3.5 ^{d}
F3D2	71.9 ± 2.1 ^{a}
F3D3	51.7 ± 1.5 ^{c}
F4D1	85.1 ± 2.0 ^{d}
F4D2	73.4 ± 2.7 ^{a}
F4D3	53.3 ± 1.0 ^{c}

* Non-identical superscript letters indicate a significant difference at P < 0.01.

Table 8. Meat yield reduction of oysters after depuration process in all treatments

TREATMENT	% reduction (Mean ± SD)*
F1D1	0.75 ± 0.4 ^{a}
F1D2	1.5 ± 1.6 ^{a}
F1D3	4.9 ± 6.7 ^{a}
F2D1	16.2 ± 1.9 ^{bc}
F2D2	19.7 ± 2.6 ^{bc}
F2D3	13.8 ± 1.9 ^{b}
F3D1	20.1 ± 2.1 ^{bc}
F3D2	21.7 ± 4.9 ^{c}
F3D3	16.8 ± 4.3 ^{bc}
F4D1	20.9 ± 4.5 ^{bc}
F4D2	19.4 ± 5.0 ^{bc}
F4D3	21.7 ± 4.8 ^{bc}

* Non-identical superscript letters indicate a significant difference at P < 0.01.

3.7. Effectiveness of the Recirculating Depuration System

Depuration of shellfishes requires additional production costs which could be the reason why some shellfish producers do not invest into this [44]. However, it is suggested that depuration promotes the development of economic activities aiming towards better utilization of bivalve molluscs [45]. They further argued that improving the microbial quality of oysters increases the commercial value of the product. Depuration is one the most effective method of reducing bacteria in shellfishes requiring less time of purification than relaying which took at least 2 months [21]. The effectiveness of depuration process depends on the design of the set-up, species [46], physiological condition [44], initial concentration of bacteria [21], water temperature and salinity in the tanks [20]. Every unique design of depuration set-up requires appropriate purification condition such as flow rate and density that is suitable for the design.

This research study has developed a small scale UV-treated recirculating depuration system that effectively reduces pathogenic bacteria in oysters (*Crassostrea iredalei)*. Furthermore, the result of this study could serve as baseline information for future studies that will assay other factors such as temperature and salinity in order to establish optimal purification condition to completely eliminate pathogenic bacteria in oysters.

4. Conclusion

This study preliminary proved the effectiveness of the small scale depuration system design for the reduction of pathogenic bacteria to improve the microbial quality of oysters as well as increasing its market value. Furthermore, the result of this study could serve as baseline information for future studies that will assay other factors such as temperature and salinity in order to establish optimal purification condition to completely eliminate pathogenic bacteria in oysters.

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