Kasetsart J. (Nat. Sci.) 42 : 503 - 512 (2008)

Application of an Electrolytic Water Treatment Technique in a *Litopenaeus vannamei* (Boone, 1931) Closed-Hatchery System

Wara Taparhudee[1]*, Saowarat Suksamran[1], Prapansak Srisapoome[1] and Niti Chuchird[2]

ABSTRACT

The application of an electrolytic water treatment technique in a Pacific white shrimp (*Litopenaeus vannamei*) closed hatchery system was investigated in three experiments. The first experiment studied the chlorine concentrations produced from an electrolytic system with different electric current and water salinity levels in a static water system. The second experiment studied the optimal electric currents and water flow rates for water treatment. The last experiment compared the closed hatchery system with an electrolytic system to an open system using chlorine powder for water treatment. It was found that in the static closed-water system, chlorine concentration increased with an increase of the duration of flow electric current and water salinity. While in the lotic condition, the optimal flow rate of water into the electrolytic system was 2.5 l/min and produced 11.4 mg/l of chlorine with an applied amperage of 1.6 A. This level was suitable for water treatment and could be applied in the *Litopenaeus vannamei* closed hatchery system for at least three crops. Although the system could reduce the usage of chlorine powder, it had a lower survival rate and a greater nitrite-nitrogen level than the control.

Key words: electrolysis technique, water treatment, *Litopenaeus vannamei*, closed hatchery system

INTRODUCTION

Thailand is an agricultural country, where the majority of people depend on agriculture for their livelihood. Aquaculture is also one of the occupations that has created a lot of wealth for the nation and the aquaculturists. In 2004, Thailand produced 251,697 metric tons of Pacific white shrimp (*Litopeneaus vannamei*) and 106,884 metric tons of black tiger shrimp (*Penaeus monodon*) and earned around 2 billion US dollars from shrimp exports (DOF, 2004). Due to the increasing world-wide demand and poor production management, many problems have occurred both in the hatcheries and in the rearing ponds, *e.g.*, low survival rates, slow growth rates, different sizes and diseases (Liu *et al.*, 1996; Alvarez *et al.*, 1998; Lavilla-Pitogo *et al.*, 1998). The causes of these problems may be due to genetics, water pollution and viral outbreak *etc*. Thus, many methods such as water treatment and closed systems have been tried to solve such problems in the ponds and hatcheries. However, the chemical costs associated with these methods,

[1] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
[2] Department of Fisheries Biology, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: ffiswrt@ku.ac.th

Received date : 18/09/07 Accepted date : 08/02/08

such as for chlorine powder and formalin, are generally costly and have to be imported. Finding new techniques in closed-hatchery systems could lead to increased production, decreased operating costs and reduced environmental problems. In the laboratory, electrolysis in saline water produces chlorine according to the equations shown below:

$$\text{Cathode: } 2Na^{+} + 2e^{-} \longrightarrow 2Na$$

$$\text{Anode: } 2Cl^{-} \longrightarrow Cl_2 + 2e^{-}$$

The chlorine produced is able to eliminate fungi, protozoa, viruses, bacteria and reduce the chemical oxygen demand and plankton levlels in the laboratory (Yoshimizu *et al.*, 1998; Tsuzuki *et* al., 1999; Jorquera *et al.,* 2002; Whangchai *et al.*, 2003a, Whangchai *et al.*, 2003b). This technique, known as electrolytic disinfection of seawater, may also be beneficial in fish culture, by increasing survival rates and also the number of fish that may be maintained in a given volume of water. It has also been able to reduce the need for antibiotics, as well as reducing the maintenance cost of pumping systems (Anonymous, 2000). Moreover, Yoshimizu *et al.* (1998) suggested that electrolytic treatment in the disinfection of seawater was as efficient as ozonation and UV treatment, but it had the advantage of being able to disinfect larger volumes of hatchery effluents, compared with the other two methods. Therefore, it was considered worthwhile to apply this technique in order to disinfect waste effluents and the water supply and to prevent diseases and pathogenic contamination in a shrimp hatchery system and also to the environment.

MATERIALS AND METHODS

The study was carried out at the Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. It comprised three experiments. The first experiment studied the chlorine concentration produced from different electric currents and different water salinity levels in a lentic condition. In an electro-oxidation experiment, two electrodes made of titanium-based metals coated with TiO_2 and an aluminium alloy, were used as a cathode and an anode, respectively. The anodic and cathodic electrodes were situated three cm apart and dipped in the saline water to a depth of about 1.5 cm. The total effective surface area of the electrodes was 9 cm^2. An AC/DC converter (DC power supply HY 3020) was used to convert 220 VAC to 1 to 24 VDC. Electric currents used were 0.1, 0.2, 0.4, 0.6 and 0.8 A. Two liters of saline water at salinities of 5, 10, 15, 20 and 30 ppt were used. During the experiment, the water in a 2.5 liter beaker was continuously stirred using a magnetic stirrer. Free chlorine in the water was analyzed by the standard method (APHA *et al.*, 1995).

The second experiment aimed to find a suitable electric current and flow rate for the water treatment. A 1,000 liter tank, with 30 ppt saline water, (normal for shrimp hatcheries) was given flow rates of 2.5, 3.0 and 4.0 l/min. Electric currents at 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 A, were applied to the electrodes as the water flowed by (Figure 1). Free chlorine in the water was analyzed by standard method (APHA *et al.*,1995). A chlorine concentration of 10 mg/l was recommended for water treatment (Worasing and Chanratchakool, 1996).

The last experiment compared the closed electrolytic system with the open chlorine-powder system. A completely randomized design was applied for this experiment. The experiment involved nursing shrimp from nauplii to the post larvae 15 stage. Shrimp nauplii were raised in six 500 liter fiber glass tanks. Three tanks were operated in a closed system with electrolysis, based on the results of the second experiment (Figure 2). The other three tanks were operated using an open system with chlorine powder for water treatment. The zoea 3 stage were fed by *Chaetoceros* spp. at a density of 100,000 cell/cc five times a day. Mysis 1 to mysis 3 stages were

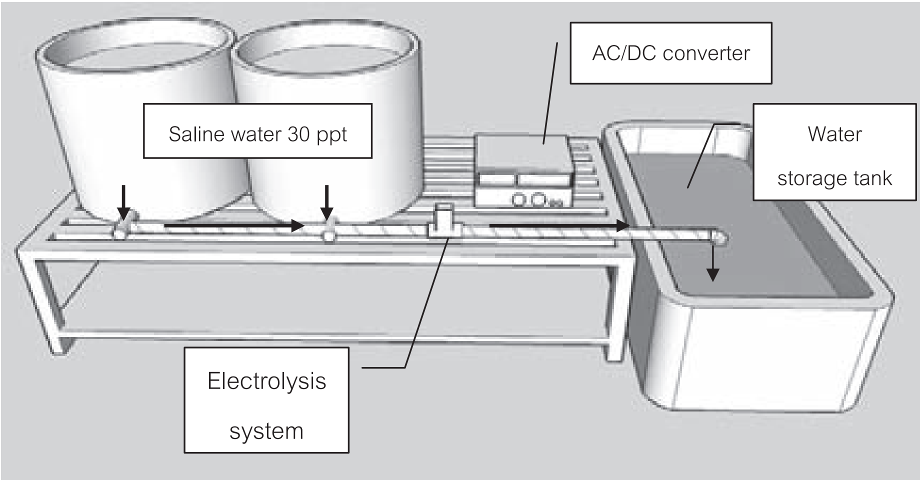


Figure 1 Diagram of electrolysis application.

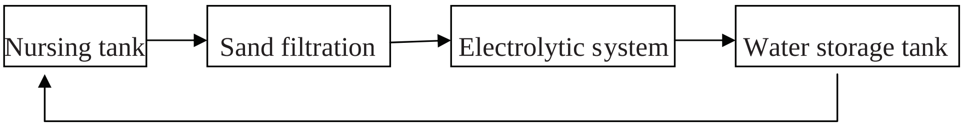


Figure 2 Closed system with electrolysis.

fed with *Chaetoceros* spp. and *Artemia* spp. five times a day and the post larval stage was fed with *Artemia* spp. five times a day. Water exchange began at the mysis 1 stage, at a rate of 25 to 30% of the total water volume in the tank every two days. Water effluent from the closed system was passed through the electrolytic system for water treatment and prepared for reuse. This nursing system was operated over three crop cycles to study the feasibility of the closed system using electrolysis. During the experiment, water qualities such as dissolved oxygen, pH, salinity, temperature, conductivity, total ammonia, nitrite-nitrogen, free chlorine, total bacteria count and *Vibrio* spp. count were monitored weekly.

The electrolytic system was supplied by an AC/DC converter (DC power supply HY 3020) reducing the power supply from 220 V AC to 1 to 24 V DC. Water quality parameters such as pH, dissolved oxygen, conductivity, salinity and temperature were measured using a TOA water quality checker (WQC-20A). Total ammonia was analysed using an indophenol method (Grasshoff, 1976) and nitrite-nitrogen was analysed by a diazotization method (Grasshoff, 1976). Free chlorine was measured by the standard method (APHA *et al*., 1995). Counts of colony forming units (CFU) were done by the total plate count method and the number of *Vibrio* spp. was counted using thiosulphate-citrate-bile salts-sucrose (TCBS) agar (APHA *et al*.,1995).

Shrimp growth and survival rates were calculated weekly. A t-test was used to determine any significant differences ($P<0.05$) between survival rates, shrimp sizes, water quality parameters, total numbers of bacteria and *Vibrio* spp. in the two treatments. The operating costs, e.g. the cost of depreciation, nauplii, artemia, saline water, chlorine powder, labor and electricity were also calculated. The production cost was defined as the total operating costs divided by the number of post larvae.

RESULTS AND DISCUSSION

Chlorine concentration produced from different levels of electricity and water salinities in the static condition

The results showed that chlorine concentration increased as duration of flow, electric current and water salinity increased (Figure 3a to 3f).

Suitable flow rate and electric current to produce chlorine at the water treatment concentration

The combination of three levels of flow rates at 2.5, 3.0 and 4.0 l/min and 9 electric currents at 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 A.DC for a 30 minute period were examined. It was found that increasing the flow rate reduced the chlorine concentration, while increasing the

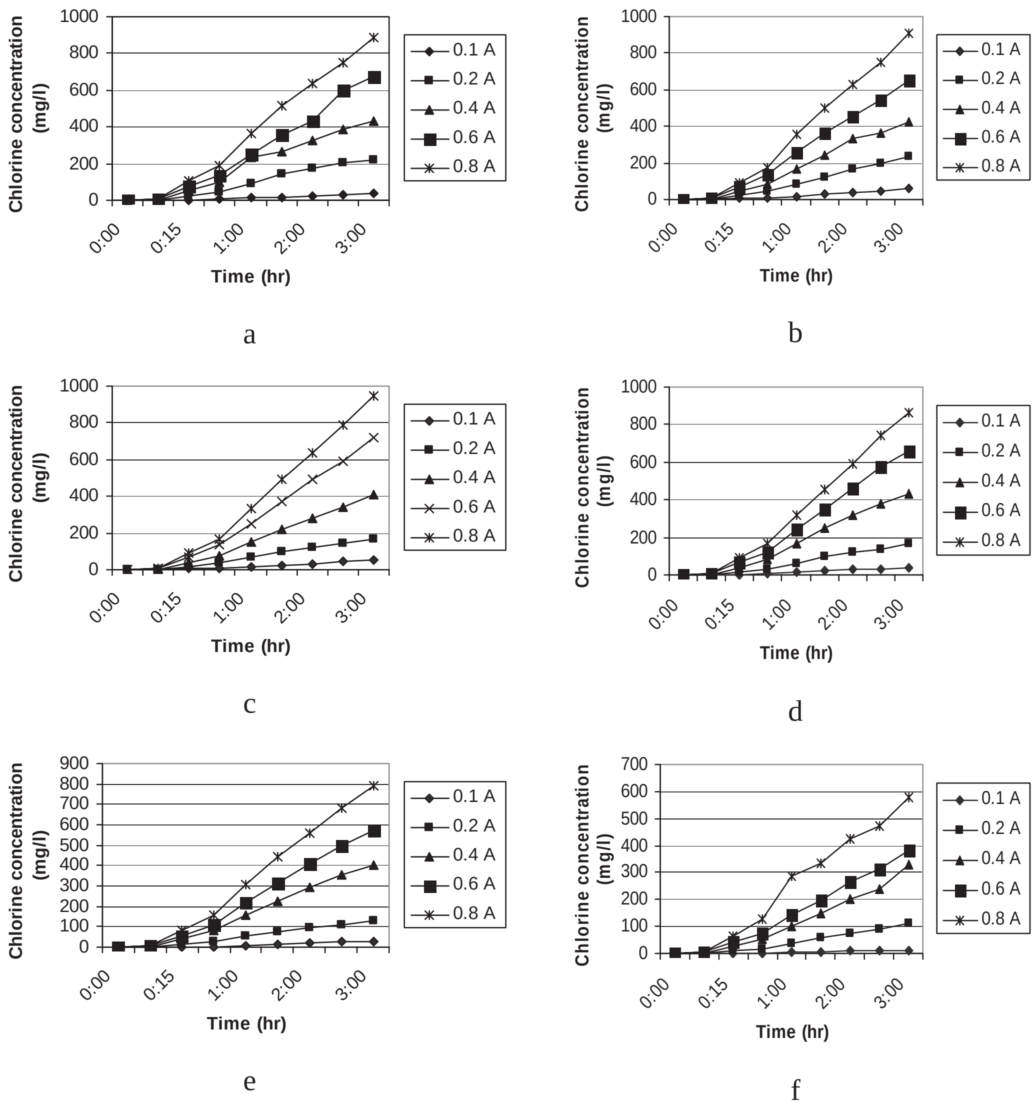
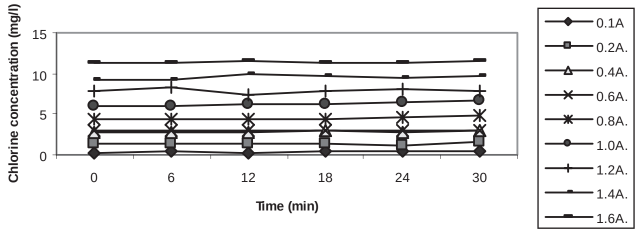


Figure 3 Chlorine concentrations (mg/l) produced by an electrolytic system at different electric currents of 0.1, 0.2, 0.4, 0.6 and 0.8 A.DC with water salinity of (a) 5 ppt, (b) 10 ppt, (c) 15 ppt, (d) 20 ppt, (e) 25 ppt and (f) 30ppt.

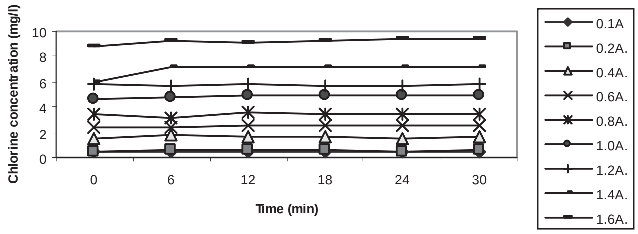
electric current increased the chlorine concentration (Figure 4a to 4c). The most suitable flow rate and electric current were 2.5 l/min and 1.6 V.DC, respectively, which produced 11.19 to 11.59 mg/l of chlorine. Worasing and Chanratchakool (1996) reported that at a chlorine concentration of 10 mg/l the growth of bacteria and *Vibrio* sp was inhibited within 30 min and there was no increase in bacteria level within 24 hr.

Comparison between a closed system with electrolysis and an open system

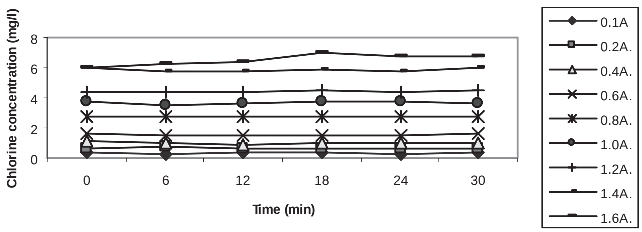
Water treatment using chlorine powder and electrolysis showed a similar effect regarding the reduction of bacterial levels (*Vibrio* spp. and total bacteria) as shown in Table 1. The bacteria



a



b



c

Figure 4 Chlorine concentration (mg/l)produced by a electrolytic system in water with a salinity of 30 ppt at different electric currents of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 A.DC, and at different flow rates of (a) 2.5, (b) 3.0 and (c) 4.0 l/min.

Table 1 Viability of bacteria in hatchery water supply after treatment using different methods of disinfection.

Treatment method	*Vibrio* sp. (CFU/ml)	Total plate count (CFU/ml)	Reduction rate (%)
Non-treated	1.55×10^2	6.1×10^4	-
Chlorine powder	0	0	>99.9
Electrolysis	0	0	>99.9

in the used water were eliminated using an electrolytic treatment (Table 2). The hypochlorus acid and hypochlorite ion produced from the electrolytic system were powerful oxidizing agents that destroyed bacteria by diffusing through cell walls and killed by inactivating certain enzymes (Lawson, 1994; Beer, 2000 and Keith, 2000).

Most water quality parameters of the treatments were not significantly different (P>0.05). There was an exception for pH in the second crop in the open system which was significantly higher than in the closed system (P<0.05) and for nitrite-nitrogen in the third crop in the closed system which was significantly higher than that of the open system (P<0.05) (Table 3). However, the trend of water quality in the closed system became worse as the number of crop cycles increased. This was probably due to the fact that level organic matter in the system, thus the nitrite-nitrogen and the number of bacteria increased, as the number of crop cycles increased.

Survival of PL15 in the closed electrolytic system was lower than the open system but there was not much difference (P>0.05). However, shrimp weight and length in the first crop and shrimp weight in the third crop were significantly different at P<0.05 (Table 4). The weight and length of the shrimp in the closed system with electrolysis were greater as the survival rate was lower. The cause of the low survival rate may have been due to the fact that after the water passed through the electrolytic system, the pH and alkalinity decreased due to hypochlorus acid (HOCl) and hypobromus acid (HOBr) reacting with ammonia and producing H^+ (Anonymous, 2000). The pH and alkalinity levels were important to molting and shell formation of the shrimp. Another reason was a high level of organic matter in the closed system that caused high nitrite-nitrogen levels and increase the level of *Vibrio* spp. along with other bacteria, which could adversely affect the shrimp larvae. Moreover, after HOCl and OCl^- reacted with ammonia, it would produce amine compounds as the equations shown below:

$$NH_3 + HOCl \longrightarrow NH_2Cl \text{ (monochloramine)} + H_2O$$

$$NH_2Cl + HOCl \longrightarrow NHCl_2 \text{ (dichloramine)} + H_2O$$

$$NH_2Cl + HOCl \longrightarrow NCl_3 \text{ (trichloramine)} + H_2O$$

The di- and trichloramines are toxic than free chlorine and monochloramine and their residuals are difficult to remove which may affect the shrimp larvae (Lawson, 1994; Rajapogal *et al.*, 1997; Stauber, 1998).

Table 5 shows that operating cost of the closed system with the electrolytic system was lower than that of the open system, because of the reduced chlorine powder cost. However, the production cost of the closed system was higher than that of the open system. This was due to lower survival rates in the closed system compared to the open system.

Table 2 Viability of bacterial counts in hatchery water supply after using electrolysis.

Water reused	Electrolysis	*Vibrio* sp. (CFU/ml)	Total plate count (CFU/ml)	Reduction rate (%)
First time	Before	1.03×10^3	4.5×10^3	-
	After	0	0	>99.9
Second time	Before	3.0×10^2	6.3×10^3	-
	After	0	18	99.7

Table 3 Water quality, *Vibrio* sp and total plate count of the 1st , 2nd and 3rd crops.

Parameter	First crop					
	Control			Treatment		
Temperature (°C)	28.2	±	0.7	28.2	±	0.7
Turbidity (NTU)	9.5	±	3.0	9.7	±	2.7
Salinity (ppt)	2.7	±	0.8	2.7	±	0.7
Conductivity (S/m)	4.4	±	1.1	4.3	±	1.1
DO (mg/l)	6.8	±	0.4	6.8	±	0.3
pH	7.9	±	0.1	7.8	±	0.2
Alkalinity (mg/l)	95.0	±	18.9	87.1	±	12.9
Total ammonia (mg/l)	2.9	±	3.8	2.3	±	2.6
Nitrite-nitrogen (mg/l)	0.04	±	0.02	0.09	±	0.19
Vibrio sp.(CFU/ml)	456.7	±	1,306.5	2,339	±	4,858.3
Total bacteria plate count(CFU)	11,675.8	±	8,995.6	11,366.7	±	6,588.4
	Second crop					
	Control			Treatment		
Temperature (°C)	31.4	±	1.1	31.4	±	1.1
Turbidity (NTU)	17.7	±	9.5	12.8	±	8.1
Salinity (ppt)	2.1	±	0.9	2.3	±	0.8
Conductivity (S/m)	3.5	±	1.3	3.8	±	1.2
DO (mg/l)	6.3	±	0.3	6.5	±	0.3
pH*	7.8	±	0.2	7.6	±	0.3
Alkalinity (mg/l)	90.8	±	10.0	88.9	±	8.9
Total ammonia (mg/l)	1.4	±	1.0	1.3	±	1.1
Nitrite-nitrogen (mg/l)	0.15	±	0.11	0.18	±	0.11
Vibrio sp. (CFU/ml)	10,390.0	±	10,459.0	7,755.3	±	8,772.6
Total bacteria plate count(CFU)	88,460.5	±	229,540.4	42,353.3	±	69,199.3
	Third crop					
	Control			Treatment		
Temperature (°C)	32.0	±	1.6	31.2	±	1.1
Turbidity (NTU)	20.1	±	12.2	27.1	±	19.1
Salinity (ppt)	2.1	±	1.1	2.2	±	0.8
Conductivity (S/m)	3.5	±	1.6	3.6	±	1.3
DO (mg/l)	5.8	±	0.7	6.0	±	0.7
pH	7.7	±	0.3	7.3	±	1.9
Alkalinity (mg/l)	110.5	±	20.3	110.3	±	12.1
Total ammonia (mg/l)	1.17	±	1.20	1.17	±	1.07
Nitrite-nitrogen (mg/l)*	0.03	±	0.04	0.70	±	0.70
Vibrio sp. (CFU/ml)	56,082.7	±	114,580.5	195,727	±	475,457.6
Total bacteria plate count(CFU)	31,577.3	±	30,860.1	34,196.0	±	33,617.9

* significantly different at $P<0.05$

Table 4 Survival rate, weight and length of shrimp post larvae 15 of the first,second and third crops.

Parameter	First crop	
	Control	Treatment
Survival rate (%)	47.5 ± 6.9	32.3 ± 1.2
Weight (g) *	0.004 ± 0.001	0.012 ± 0.003
Length (mm) *	10.6 ± 0.7	13.9 ± 1.3
	Second crop	
Survival rate (%)	58.6 ± 13.0	41.2 ± 4.2
Weight (g)	0.011 ± 0.002	0.017 ± 0.002
Length (mm)	14.5 ± 3.3	13.7 ± 0.9
	Third crop	
Survival rate (%)	88.5 ± 7.3	70.5 ± 16.2
Weight (g) *	0.014 ± 0.003	0.020 ± 0.007
Length (mm)	14.5 ± 1.1	15.8 ± 2.9

* It is significantly different at $P<0.05$

Table 5 Comparison between average operating costs of open and closed systems with electrolysis.

Items	Control (Baht)	Treatment (Baht)
First crop		
Fixed costs		
- Depreciation of electrolytic system		138.88
Variable costs		
- 100,000 Nauplii	1,000.00	1,000.00
- 3 cans of Artemia cysts	850.00	850.00
- Saline water 140 ppt	333.79	333.79
- Chlorine powder	33.48	
- Labor	175.00	
- Electricity		9.00
Total	2,392.27	2,331.67
Production cost (Baht/piece)	0.050	0.072
Second crop		
Fixed costs		
- Depreciation of electrolytic system		138.88
Variable costs		
- 100,000 Nauplii	1,000.00	1,000.00
- 3 cans of Artemia cysts	850.00	850.00
- Saline water 140 ppt	333.79	
- Chlorine powder	33.48	
- Labor	175.00	
- Electricity		9.00
Total	2,392.27	1,997.88
Production cost (Baht/piece)	0.041	0.048

Table 5 Comparison between average operating costs of open and closed systems with electrolysis (cont).

Items	Control (Baht)	Treatment (Baht)
Third crop		
Fixed costs		
- Depreciation of electrolytic system		138.88
Variable costs		
- 100,000 Nauplii	1,000.00	1,000.00
- 3 cans of Artemia cysts	850.00	850.00
- Saline water 140 ppt	333.79	
- Chlorine powder	33.48	
- Labor	175.00	
- Electricity		9.00
Total	2,392.27	1,997.88
Production cost (Baht/piece)	0.027	0.028
Grand total	7,176.81	6,327.46

Remark: 1 US dollar = 34 Baht

CONCLUSION

In the electrolytic system, the chlorine concentration increased as the duration of flow, electric current and salinity levels increased. In this system, it was found that the suitable flow rate was 2.5 l/min, with 1.6 V.DC applied to the electrodes to produce chlorine at a concentration of 11.4 mg/l. This concentration level was enough for water treatment and could be used in a shrimp larval hatchery system for at least three crops. The results obtained suggested that salt water electrolysis was an efficient and comparatively low-cost alternative method for the control of pathogens in shrimp hatcheries, if it could improve the survival rate. This method would reduce the cost of using chlorine powder. However, to make the system more effective, calcium hydroxide $Ca(OH)_2$ should be added to in the water after electrolysis treatment to increase the pH and alkalinity. The filtration system should also be improved because turbidity and nitrite-nitrogen levels increased after each crop cycle due to the persense of left over feed and insufficient treatment levels. Moreover, further study should determine the effect of dichloramine and trichloramine on shrimp larvae.

ACKNOWLEDGEMENTS

The authors wish to thank the Kasetsart University Research and Development Institute for financial support with this project.

LITERATURE CITED

Anonymous. 2000. **Electrolyzed Water**. Available source: http://www.aquatechnology.net/electrolyzed.html, 25/05/07.

APHA, AWWA and WPCA. 1995. **Standard Methods for the Examination of Water and Wastewater**. 19 th ed., American Public Health Association, Washington, D.C. 1,134 p.

Alvarez, J.D., B. Austin, A.M. Alvarez and H. Reyes. 1998. *Vibrio harveyi*: a pathogen of penaeid shrimps and fish in Venezuela. **J. Fish Dis**. 21, 313–316.

Beer, C. 2000. **Recreational Water Disinfection by Coppers/Silver Ions**. Available source: http://www.clearwaterpoolsystems.com/

waterdis.htm, 13/12/2000.

DOF. 2004. **Fisheries Statistics of Thailand (CD-ROM).** The Department of Fisheries, Thailand.

Grasshoff, K. 1976. **Methods of Seawater Analysis**. Verlag Chemie, Weinheim, New York. 317 p.

Jorquera, M.A., G. Valencia, M. Eguchi, M. Katayose and C. Riquelme. 2002. Disinfection of seawater for hatchery aquaculture systems using electrolytic water treatment. **Aquaculture** 207: 213-224.

Keith, A.C. 2000. **Cloro (Spanish).** Chorine Chemistry Council Home. Available source: http://www.c3.org/chlorine_knowledge_center/whitepapers10-98.html, 11/07/ 2001.

Lavilla-Pitogo, C.R., E.M. Leano and M.G. Paner. 1998. Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent vibrios in the rearing environment. **Aquaculture** 164 (1-4): 337-349.

Lawson, T.B. 1994. **Fundamentals of Aquacultural Engineering.** Chapman&Hall. New York. 355 p.

Liu, P.C., K.K. Lee, K.C. Yii, G.H. Kou and S.N. Chen. 1996. Isolation of *Vibrio harveyi* from diseased kuruma prawns *Penaeus japonicus*. **Curr. Microbiol**. 33: 129–132.

Rajapogal, S., K.V.K. Nair, G. der Velde and H.A. Jnner. 1997. Response of mussel *Brachidontes striatulus* to chlorination: an experiment study. **Aquat. Toxicol**. 39: 135-149.

Stauber, J.L. 1998. Toxicity of chlorate to marine microalgae. **Aquat. Toxicol**. 41: 213-227.

Tsuzuki K., T. Tanaka, T. Takagi and M. Shimomura. 1999. Effect of electrochemical treatment on Microcystis extinction [Japanese]. **J. Water Environ. Soc**. 22 : 228-231.

Whangchai, N., N. Nomura and M. Matsumura. 2003a. Factors affecting phytoplankton removal by electro-oxidation of artificial seawater. **Chiang Mai J. Sci**. 30 : 255-259.

___________ . 2003b. Phytoplankton control in artificial seawater using electrolytic treatments**. Thai J. Agric. Sci**. 36 : 297-304.

Worasing, S. and P. Chanratchakool. 1996. Acute toxicity and effects of chlorine on black tiger shrimp, *Penaeus monodon* Fabricius. **Technical paper No.** 34/1996, Chanthaburi Coastal Aquaculture Development Center, Coastal Aquaculture Division, Department of Fisheries. 26 p.

Yoshimizu M., A. Ishikawa, Y. Hori, T. Yoshinaka, Y. Ezura, K. Watanabe and T. Uenishi. 1998. Disinfection of water system for aquaculture by electrolyzation of sea water [Japanese], pp. 82-83. *In* **Functional Water Symposium'98**, Functional Water Foundation, Tokyo, Japan.