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The Effects of Using Steel-Making Waste Slags as Substrates on Shrimp *Penaeus monodon* Reared in Aquaria

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Abstract

The massive pumping of underground water for shrimp (*Penaeus monodon*) farming in two areas in Taiwan has caused ground depression of 1-2 m and resulted in difficulty in draining and drying the ponds. A study was conducted to evaluate the potential of using steel-making waste slags as substrates on which to raise shrimp.

Three experiments were conducted in aquaria. Experiments I and II used six substrates: "rocky" type -blast furnace slag (BF), converter slag (BOF), concrete (CON); "granulated" type - granulated slag (GS), beach sand (BS); and "smooth" type - blank (BNK) or glass substrate. Experiment III had five substrates: BF, BOF, CON, and GS all covered with beach sand; beach sand alone served as control. The results indicated that substrate types were much more important in growth than the constituents of the water, despite the varying chemical nature of the substrate. The growth rates with substrates were significantly better than without. Shrimp growth on granulated substrates of BS and GS was significantly better than on rocky substrates of BF, BOF and CON. No significant differences in mortality were observed for the different substrates which could be either related to their physical features or chemical compositions. In Experiment III, beach sand covering the waste slags eliminated any effect of the waste slags on growth and mortality. The concentrations of Fe, Mn, Cu and Zn in muscle, viscera and shell of shrimp from different treatments were not significantly different.

Introduction

Along with the increased production of steel from the China Steel Corporation (CSC), the generation of steel slag, a waste product, has also increased. The slag amounted approximately to 900,000 t in 1986, including blast furnace slag (BF) converter slag (BOF) and water-cooled BF slag (granulated slag, G slag (GS)).

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Both BF and BOF slags are rock-like and GS is granulated. Slag generally contains calcined lime (CaO) and silica (SiO₂) as principal constituents. BF slag also contains alumina (Al₂O₃) and manganese oxide (MnO). The granulated slag, manufactured by quenching with water from the molten state, has no time to form crystals as in aircooled slag and has a vitreous structure after cooling. Its chemical composition is basically the same as BF slag. All these constituents are limited to those usually composing the crust of the earth and their chemical compositions are similar to that of average sedimentary rock and Portland cement (Table 1). GS has been used in making tiles. BF and BOF slags are used as road construction materials or discarded along the coast, their value as a resource not being appreciated. It has been CSC's goal to expand and diversify the use of slags.

	Constituents (unit: % in weight)											
	SiO2	CaO	MgO	Al203	FeO	MnO	TiO2	S				
BF*	84-37	36-41	7-9	14-16	0.3-0.8	0.5-0.8	0.4-0.6	0.4-0.7				
BOF*	13-16	40-52	4-7	0.7-1.2	*	3.5-5.0	0.4-0.6	0.08-0.18				
Pit soil** Cement**	59.6 22.0	0.4 65.1	0.8 1.3	22.0 5.2	# #	0.1 #	*	0.01 1.8				

Table 1. Comparison of chemical position of steel slags (BF and BOF), pit soil and Portland cement.

*Data from Department of Research Development, China Steel Corporation. **Data from Kato (1962).

#Compound not quantified.

In Cha-Tung and Lin-Pen area, which is approximately 30 km southeast from the CSC, massive pumping of underground water for shrimp (*Penaeus monodon*) farming has caused ground depression of 1-2 m and resulted in difficulty in draining and drying ponds. It is a common practice among shrimp farmers to routinely vary the salinity between 15 and 20 ppt in shrimp ponds to stimulate shrimp molting and consequently to increase growth. It has been estimated for a stocking density between 150,000 and 270,000 shrimp ha-1, freshwater consumption is 31,500 to 125,000 t \cdot ha-1 (Hsu and Chang 1984).

To compensate for the ground depression CSC has proposed to fill the shrimp ponds with steel slag to elevate the bottom level which would not only benefit the shrimp farmers but create a way of disposing of CSC's waste products. The purpose of this study was to evaluate the possible use of slags as substrates on which to raise shrimp, and to monitor heavy metal accumulation in shrimp tissues.

Materials and Methods

The study was conducted at the Department of Aquaculture, National Taiwan College of Marine Science and Technology (NTCMST). Three experiments were conducted.

Six substrates of three types were evaluated in duplicate in Experiments I and II: "rocky" - BF, BOF and concrete blocks used in pond dikes (CON); "granulated" - GS and beach sand (BS); and "smooth" - no substrate (BNK) or glass substrate. The beach sand served as control. The substrates were spread evenly to a thickness about 5 cm in 75 x 30 x 40 cm³ aquaria. The water was maintained at a salinity around 15 ppt and at a depth of 25 cm in all aquaria. The seawater was pumped from the adjacent beach and filtered through a sand bed before use.

The rearing duration of Experiment I was six weeks. Shrimp used were obtained from a farm and were stocked at a density of four shrimp per aquarium (18 m⁻²). The average size at stocking was 11.7 g. The average water temperature was 27°C. Experiment II was conducted for 12 weeks. The shrimp were of similar size to that of Experiment I and stocked at a density of six shrimp per aquarium (27 m⁻²). The water temperature averaged 21°C.

Five substrates were used in Experiment III; BF, BOF, CON and GS, which were covered with beach sand (BF+bs, BOF+bs, CON+bs and GS+bs), and beach sand alone (BS) served as control. This arrangement was to eliminate the effects of the physical features of the substrate. Each treatment was also in duplicate. The stocking rate was six shrimp per aquarium (27 m⁻²) and the average size of shrimp was 11.8 g. Average water temperature was 24°C. Experiment III lasted for eight weeks.

The aquaria were filled two weeks before the shrimp were stocked. Water was completely drained after one week's submergence. Before draining, water was sampled for heavy metal content analysis. One-third of the water was changed weekly, including the week before shrimp were stocked. The exchange rate was similar to that in the production pond, 5 to 10% daily or every two days. Every week before changing the water from the aquarium, 0.5 l of water was sampled for analyses. Analyses were made for NO_3 -N (Grasshoff-Wood method), NO_2 -N (Wood-Armstrong- Richard method), PO₄-P (molybdenum blue-ascorbic method), S²-, SiO₂ (molybdosilicate method), pH (Photovolt 126 A pH meter), and heavy metals (Fe, Mn, Cu, Zn, Cd, Ni, Co and Cr for Experiment I and only Fe and Mn for Experiment II). Heavy metals were monitored with an atomic absorption spectrophotometer (HITACHI, model 170-40) by the direct salt matrix correction methods (Chen and Pai 1985). The detection limit of each heavy metal was as follows: Mn, Cd and Zn 0.01 mg l⁻¹, Ni 0.02 mg l⁻¹, Cu 0.04 mg l⁻¹, Fe and Co 0.06 mg l⁻¹, and Cr 0.08 mg l⁻¹.

Shrimp were weighed to the nearest 0.1 g every two weeks. Commercial shrimp feed (Wanyng brand) was fed to the shrimp at 5% average body weight at 0830 and 1730 hours. Since the period of the experiments was not long enough to show the sigmoid or decaying exponential growth phenomenon (Ricker 1975), a simple linear regression equation $Y = B_0 + B_1X$, where Y is the shrimp weight in g, X time in weeks and B_1 the growth rate in g week⁻¹, was used.

When stocking, the rostrum of each shrimp was sheathed with a piece of tiny plastic tubule. Dead shrimp were replaced by unsheathed individuals of similar size to maintain stocking density and the growth rates were corrected by the difference of the dead shrimp and the replaced shrimp. The weekly instantaneous mortality z was calculated by solving the equation $S_t = S_{t-1} \cdot \exp(-z \cdot t)$ (Laurence 1974; Heinrich 1981) where S_t and S_{t-1} are the survival rate at weeks t and t-1, respectively; $S_t = 1$ - (total number of dead shrimps up to the week t period/total number of shrimp stocked up to week t).

At the end of Experiment II, two sheathed shrimp from each a quarium were analyzed for Fe, Mn, Cu and Zn content in shell, muscle and viscera using standard methods (APHA 1981). The samples were first digested with HNO_3 -HClO₄ (Greenberg et al. 1981) then were analyzed with an atomic absorption spectrophotometer.

The differences in the growth rate and in heavy metal content of shrimp among treatments were tested by one-way analysis of variance. In Experiments I and II, the sum of squares for treatments were subdivided to form an orthogonal comparison set (Neter and Wasserman 1974) to test the differences of growth rates and mortality of shrimp for (1) with - without substrate; (2) rocky granulated substrate; (3) between two granulated substrates; (4) between steel slags and concrete block; and (5) between two rocky slags. In Experiment III, Duncan's Multiple Range Test was conducted to find out the differences of growth rates and mortality of shrimp between treatments.

Results

Growth

In Experiment I the average growth rates (g week-1) on granulated substrates BS (1.27) and GS (1.09) were significantly (P < 0.05) superior to those on rocky substrates CON (0.74), BOF (0.71) and BF (0.47) (Table 2). Growth in treatments with substrates was

Table 2. The analysis of variance with orthogonal comparison of the growth rate (g week-1) of shrimp *Penaeus monodon* cultured in aquaria over six substrates (see text for acronyms) for (a) six weeks in Experiment I and (b) 12 weeks in Experiment II. The Experiment III (c) for eight weeks had five substrates: BF, BOF, CON and GS covered with beach sand: (BF+bs, BOF+bs, CON+bs and GS+bs) and beach sand (BS) alone as control. Duncan's Multiple Range Test, instead of orthogonal comparison, was conducted for Experiment III. No significant differences among treatments were found so the ANOVA table was not listed.

(a) Experiment I

Treatme	at	BNK	G8	B 8	B P	BOP	CON		1170.000			
Replicate	1 2	0.46 0.39	1.01 1.18	1.35 1.19	0.42 0.58	0.74 0.98	0.87 0.81					
Average		0.87	1.09	1.27	0.47	0.71	0.74					
ANOAY	Table					100						
Source of	variatio	D							DF	88	MS	y
Treatme	at					1-5654.0			5	0.2024	0.0405	6.22*
			BNK	BS	GØ	BF	BOF	CON				
Non-sub	ve. sub		5	4	-1	4	4	-1	1	0.1118	0.1118	1738*
(BNK ve.	B8, G8,	BF, BOF	and CON	n								
Sandy pe	Rocky		0	8	8	-8	-2	-2	1	0.0680	0.0680	10.45°
(BS and	38 os. B	F, BOF a	nd CON)									
G8 pc. B	5		0	1	-1	0	0	0	1	0.0016	0.0016	0.95ms
Slag us. (CON		0	0	0	1	1	-2	1	0.0000	0.0000	0.00ns
(BF and I	BOF pe.	CON)										
BP ve. B)F		0	0	0	1	-1	0	1	0.0210	0.0910	3.23m
Errar									6	0.0391	8800.0	
Total									11	0.9415		
(ь)	Experin	nent II										
Trestme	at	BNK	G8	BS	BF	BOF	CON					
Replicate	1	0.05 0.15	0.57 0.40	0.54 0.85	0.20 0.25	0.37 0.37	0.32 0.27					
Average		0.10	0.48	0.44	0.22	0,37	0.29	27				

continued

Table 2 (continued)

ANOVA Table

			_							
Source of variation							DF	88	MS	F
Trestment							5	1.2058	0.2411	24.88**
	BNK	BS	GS	BF	BOF	CON				
Non-anb ps. sub	5	-1	-1	-1	1	-1	1	0.3868	0.3868	89.81**
(BNK vs. BS, GS, BF, BOF	and CON	•								
Sandy os. Block	0	8	8	-2	-2	-2	1	0.7020	0.7020	70.97**
(BS and GS vs. BF, BOF an	ad CON)									
GS m. BS	0	1	-1	0	0	0	1	0.0806	0.0806	8.10ms
Sing vs. CON	0	0	0	1	1	-2	1	0.0290	0.0290	2.98ns
(BF and BOF or. CON)										
BF ve. BOF	0	0	0	1	-1	0	1	0.0552	0.0552	5.58ns
Error							6	0.0594	0.0099	
Total							11	1.2650		
(c) Experiment III										
Treatment	GS+bs	BS	BF+ba	BOF+be	CON+ba	1				
Replicate 1 2	0.95 0.89	1.19 1.14	1.25 1.16	1.06 0.80	1.12 1.08					
Avenge	0.92	1.17	1.21	0.98	1.07					

*Significant (P < 0.05). **Highly significant (P < 0.01). ns - Not significant (P > 0.05).

highly significantly (P< 0.01) faster than without substrate. No differences were detected between BS and GS or among BF, BOF, and CON. The results from Experiment II also showed best growth on granulated, medium growth on rocky and the poorest growth on blank substrates (Table 2). There were also no differences between BS and GS or among BF, BOF and CON. The results of statistical tests agreed with those of Experiment I except the significant levels were all with P < 0.05; none with P < 0.01. Lower water temperature and higher stocking density in Experiment II generally resulted in slower shrimp growth than in Experiment I.

In Experiment III, the average growth rates (g week-1) were BF+bs (1.21), BS (1.17), CON+bs (1.07), BOF+bs (0.93) and GS+bs (0.92) (Table 2). No significant differences (P > 0.05) in growth rate were found among the five treatments.

Mortality

In Experiment I, weekly instantaneous mortality (Z) was the lowest in GS treatment (0.095) and highest in BF (0.175) and BNK

(0.19) (Table 3). In Experiment II, GS (0.022) and BS (0.027) treatments had the lowest mortality and BOF (0.077) and BNK (0.076) had the highest mortality. No significant differences in mortality were found among treatments in both Experiment I and II.

In Experiment III, the average mortalities were BS (0.073), GS+bs (0.073), CON+bs (0.084), BOF+bs (0.087) and BF+bs (0.09) (Table 3). No significant differences in mortality were found among the five treatments.

Table 3. The analysis of variance with orthogonal comparison of the weekly instantaneous mortality of shrimp *Penaeus monodon* cultured in aquaria over six substrates (see text for acronyms) for (a) six weeks in Experiment I and (b) 12 weeks in Experiment II. The Experiment III (c) for eight weeks had five substrates: BF, BOF, CON and GS covered with beach sand: (BF+bs, BOF+bs, CON+bs and GS+bs) and beach sand (BS) alone as control. Duncan's Multiple Range Test instead of orthogonal comparison, was conducted for Experiment III. No significant differences among treatments were found so the ANOVA table was not listed.

(a)	Experiment I										
Treatmen	e BNK	GS	B 8	BF	BOF	CON					-
Replicate	1 0.21 2 0.17	0.18 0.06	019 018	0.20 0.16	0.18 0.14	0.11 0.21				210 - 11 DAT	
Average	0.19	0.095	016	0.175	0.16	0.16					
ANOVA T	able					1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 - 1945 -					
Source of	variation							DF	88	MS	F
Tretmen	•					715977	10	5	0.0057	0.0011	8.28ns
		BNK	BS	G9	BF	BOP	CON				
Non-sub v	e sub	5	-1	-1	-1	-1	-1	1	0.0019	0.0019	5.60ns
(BNK va. 1	88, 08, BF, BC	F and COP	Ð								
Sandy ve.	Rocky	0	8	8	-2	-2	-2	1	0.0020	0.0020	5.66 na
(BS and G	8 14. BF, BOF	and CON)									
GS ve. BS		0	1	1	0	0	0	1	0.0000	0.0000	0.06.00
Slag pe. C	on 🧧	0	0	0	1	1	-2	1	0.0004	0.0004	1.11ns
BP and B	OF us. CON)										
B F va. BO)	7	0	0	0	1	-1	0	1	0.0014	0.0014	8.95ns
Error								6	0.0021	0.0003	
Total								11	0.0078		
(ь) 1	Experiment II										
Treatment	BNK	GÐ	BS	BF	BOF	CON					
Replicate 2		0.019 0.026	0. 019 0.035	0.053 0.028	0.065 0.090	0.019 0.065					
Average	0.076	0.022	0.027	0.040	0.077	0.042					

(a) Experiment I

continued

Table 3 (continued)

ANOVA Table

Source of variation							DF	88	MB	7
Treatment							5	0.0108	0.0021	1.05a
	BNK	BS	GS	BF	BOF	CON				
Non-sub zs. sub	5	-1	-1	-1	-1	-1	1	0.0027	0.0027	1.820
(BNK vs. BS, GS, BF, BO	F and CON)								
Sandy ve. Block	0	8	8	-2	-2	-2	1	0.0084	0.0034	1.67m
(BS and GS os. BF, BOF a	and CON)									
GS cs. BS	0	1	-1	0	0	0	1	0.0042	0.0042	210m
Sing us. CON	0	0	0	1	1	-2	1	0.0001	0.0001	0.04m
(BF and BOF 10. CON)										
BF vs. BOF	0	o	0	1	4	0	1	0.0002	0.0002	0.11m
Error							6	0.0021	0.0008	
Total							11	0.0078		
(c) Experiment III										
Treatment	GS+bs	BS	BF+be	BOF+be	CON+bs					
Replicate 1	0.064	0.069	0.082	0.087	0.067					
2	0.062	0.077	0.101	0.087	0.101					
Average	0.073	0.073	0.092	0.087	0.084					

gnificant (P < 0.05). lighly significant (P < 0.01). - Not significant (P > 0.05).

Water Chemistry

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Manganese: In Experiments I and II. Mn concentrations were 0.15-0.17 mg l-1 in BOF and 0.12-0.13 mg l-1 in GS one week before stocking and tapered off to lower than 0.02-0.03 mg l-1 in both treatments three weeks after shrimp stocking (Fig. 1). In Experiment III, except BS, Mn concentrations in the other four treatments were lower one week before stocking than those at the following week since overlying beach sand blocked the release of Mn. In all three experiments one week before shrimp stocking. Mn concentration in BOF which contains the most Mn0, was the highest among all treatments. In Experiments I and II, although GS contained far less MnO than BOF, it had a larger contact surface with the water allowing more Mn to dissolve.

Iron: Iron concentrations tapered off as in the case of manganese (Fig. 2). Having the same chemical constituents as BF but larger surface area, iron concentrations were higher in GS than BF by the







first two weeks. BF and GS treatments had the highest iron concentration since both substrates contained iron. Lower concentrations in Experiment III were due to the beach sand which slowed down the release of iron.

Copper: In Experiment I, BF and BOF treatments had Cu concentrations of 0.068 mg l-1 during the first week before stocking shrimp. No Cu was detectable in the other treatments.

Cadmium, nickel, cobalt, lead, cromium, zinc: None of these metals were detectable in any treatment in Experiment I and therefore not measured in Experiment II and III.

pH: The average pH ranged between 7.5 and 8.4 (Fig. 3). The substrate with BOF had the highest pH in all three experiments and BNK had the lowest in Experiments I and II.



Fig. 3. Average pH, silicate, phosphate and sulfide concentration in water cultured with ahrimp *Penaeus monodon* on the six substrates described in the text.

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Silicate: In all three experiments the substrate with BF had the highest silicate concentration, 3.5 to 3.8 mg l⁻¹ (Fig. 3). In Experiments I and II, substrates consisting slag; BF, BOF and GS had higher silicate concentration than the other treatments and BNK had the lowest.

Phosphate: Average and variation of phosphate concentrations were generally higher in Experiment II than Experiments I and III (Fig. 3). Differences of average phosphate concentrations among treatments in Experiment III were not that pronounced as those in Experiments I and II. GS had the lowest phosphate concentration of 62 and 90 mg l-1 in Experiments I and II, respectively.

Sulfide: Average sulfide concentration was less than 75 mg l^{-1} in all treatments and in all three experiments (Fig. 3). In Experiment II, BNK had the highest average (66) and variation (34) of sulfide concentration. BF and BOF generally had the lowest sulfide concentration in all three experiments.

N-species: In general, the average concentrations and variations of NO_3 -N, NO_2 -N, and NH_3 in Experiment II were higher than Experiment I and III (Fig. 4). In Experiment III, when the substrates



Fig. 4. Average nitrate, nitrite and ammonia concentration in water cultured with shrimp *Penaeus monodon* on the six substrates described in the text.

were covered with beach sand, all five treatments had similar average concentrations of all three N-species. In Experiments I and II, both average and variation of all three N-species concentration in BNK were the highest among all treatments and in either GS or BS the lowest. There were no significant differences of Fe, Mn, Cu and Zn concentration ($\mu g \cdot g^{-1}$ tissue wet weight) in either muscle, viscera or shell between shrimp cultured on the six substrates (Fig. 5).



Fig. 5. The concentration of Fe, Mn, Cu and Zn in muscle, shell and viscera of shrimp *Pengeus monodon* cultured on the six substrates described in the text.

Discussion

The results from Experiments I and II suggest that physical features of substrates affect growth. Growth of shrimp with no substrate was slower than that with substrates. The shrimp in the treatment of no substrate were often found submerged in excess feed and their metabolic waste sediment. This light sediment might have inhibited feed attraction and made the search for food difficult. Furthermore, frequent contact with toxic substances such as NO₂ and NH₃ of the decaying left-over feed and feces could slow growth. Slower growth on the rocky substrates than on granulated substrates might be due to feed falling into the crevices among substrate lumps or because shrimp spent more energy searching for feed. Von Olst et al. (1975) found the juvenile lobster Homarus americanus grew larger

on sand substrate than on oyster shell, PVC tubes or rock. Furness and Aldrich (1979) found brown shrimp (*Penaeus aztecus*) growth was positively correlated with pond bottom softness. They hypothesized two mechanisms which might underlie the relationship: first, the shrimp may have expended less energy burrowing in soft substrates and consequently had more energy available for growth. Second, shrimp might have found more or better food in the softer bottoms. Quick and Morris (1976) also postulated that the soft bottoms might have allowed more "normal shrimp behavior". Shrimp grown in the treatment of no substrate were often found gliding on the glass bottom which also prevented their normal behavior.

Water chemistry seemingly had less effects on growth than did the physical features of the substrates. Constant aeration and changing the water kept water quality in all treatments below critical levels. Changing the water probably also masked the substrate effects on water chemistry. Furthermore, the highly oxidized steel slags products generally show chemical stability and released minerals and nutrients not significantly faster than the other substrates, concrete and beach sand.

It was expected the rocky substrates, BF, BOF and concrete, might provide more shelter than smoother substrates and thus reduce cannibalism. Willis et al. (1976) concluded that habitat complexity is a requirement for *Macrobrachium* sp. high density grow-out systems. According to Sandifer and Smith (1976), the most promising approach to the mass rearing of *Macrobrachium* is providing a large surface area of shelters in the tank. However, the low density in Experiment I and the low water temperature in Experiment II lessened the probability of encounters and minimized cannibalism, thus reducing the substrate configuration effects on survival of the shrimp.

There appeared to be no appreciable heavy metal accumulation in shrimp grown on steel-making slags as a substrate when the water was changed at the rate used in this study. However, when considering using steel-making slags as substrate in commercial shrimp farms, attention should be paid to the effect of initial slag effluent on the environment. The initial Mn concentrations in water of BOF and GS (0.1-0.2 mg.l-1) were lower than the Japanese criterion for fisheries of 1.0 mg.l-1 (Environment Agency 1972) but higher than the USA Coastal Water Quality Criterion, 0.1 mg.l-1 (USEPA 1976). After dilution of 50% (changing one-third of water) at the second week after filling, the Mn concentration in water of BOF and GS was lower than 0.1 mg.l-1 In the first and second week after filling, the Fe concentration in water of BF and GS (0.3-0.6 mg.l-1) exceeded US Coastal Water Quality Criterion of 0.30 mg.l-1 but were lower than Japanese criterion for fisheries, 1.0 mg.l-1. The Fe concentration in water of BF and GS was lower than 0.3 mg.l-1 after the third week after filling. Covering the slag with beach sand as in Experiment III could ease the release of Mn and Fe and help the effluent meet the recommended criteria.

In this indoor and relatively short experiment, Mn and Fe did not show beneficial effect on shrimp growth and survival. However, Shigueno (1975) indicated that iron oxide was effective on shrimp culture according to the results of an experiment done by Reijiro Hirano in 1964. In that 68-day experiment, treatment of the bottom with 2 kg of iron oxide added to a 3.3-m² tank, shrimp (Penaeus japonicus) had a remarkably lower mortality rate, higher food consumption, and higher growth rate than those in an untreated tank. The iron compound prevented formation of H₂S. Engler and Patrick (1973) found that the more soluble oxidants had the greatest effects in maintaining more positive redox potentials and in delaying sulfate reduction to sulfide. The less soluble compounds (MnO2 and $FePO_4.2H_2O$) were less effective in delaying S2- production but persisted longer in preventing maximum S2- buildup. A longer experiment than this one or greater amount of slags might reveal the positive aspect of steel slag on improving the sediment condition and hence the growth and survival of shrimp.

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