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Biofiltration and biodegradation of wastewater in shrimp farms by a species of sea cucumber, *Holothuria leucospilota* (Brandt, 1835) from Chabahar Gulf

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Abstract

Biofiltration is a pollution control technique in wastewater treatment, aquaculture, and greywater recycling in which organisms such as sea cucumber and bivalves can be used. In this study, we investigated the integrated bioremediation techniques for a shrimp farm wastewater by sea cucumber, *Holothuria leucospilota* in the Chabahar Gulf, around the Oman Sea. Furthermore, parameters such as pH, DO, turbidity, NO_3^-, PO_4^{3-}, EC, and TDS were evaluated. The concentrations of these parameters were measured in four sampling stations. The results showed that there were significant differences between the parameter values in four sampling stations ($P<0.05$), the highest concentration being observed in station 3 (outlet wastewater of shrimp farm). In addition, there were significant differences in filtration rates between different periods and different density of sea cucumber ($P<0.05$), the highest filtration was observed on the 8th day, and at the highest animal density (200). The filtration rates were 8%, 52.5%, 32%, 61.8%, and 55% for EC, NO_3^-, turbidity, PO_4^{3-}, and TDS, respectively. Based on these results, *H. leucospilota* had high capacity for absorption and removal of pollutants from shrimp farm wastewater. Therefore, *H. leucospilota* is an important species for bio-filtration and improvement of wastewater quality of shrimp and fish farms.

Keywords: Biofiltration; Shrimp Farms; Sea Cucumber; *Holothuria leucospilota;* Chabahar Gulf.

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1. Introduction

Nowadays a widely used method for wastewater pollution control is biofiltration, which uses living organisms to absorb and degrade pollutants biologically (Xie *et al*., 2004). Biofiltration is used in different environments, such as shrimp and fish wastewater, traping toxic chemicals and causing micro-biotic oxidation of contaminants (Lefebvre *et al*., 2000). In 1893, biofiltration was used for the first time, as a trickling filter, for treatment of wastewater, in England and has been widely used since then for many types of waters (Wang *et al*., 2007). Biofiltration is also used for aquacultures and greywater recycling, to enhance the quality of water while cutting down water replacement (Qing *et al*., 2013). In many countries shrimp farming is becoming a profitable business in their coastal areas (Helms *et al*., 2008). One of the main concerns with shrimp farming is the increase of organic matter and nutrients in water discharges and their impact on the environment and natural resources (Brothers *et al*., 2015).

In wastewaters of shrimp aquacultures, both living and dead planktons, feed waste, faecal matter and other excretory products of the animal are usually found (Quang, 2014). Soluble nutrients, although biodegradable, can cause eutrophication in receiving water bodies. If there is inadequate flushing ability, in the areas where large numbers of shrimp farms are established, the impact may be significant (Wang *et al*., 1990). In the areas with inadequate flushing, increased sedimentation of suspended solids, turbidity, eutrophication, algal and microbial blooms and higher demand for biological and chemical oxygen has been noticed (Dame, 1993).

Various invertebrates such as Mollusca, seaweeds and Echinodermata are used to accelerate biodegradation of aquaculture farm wastes (Quang, 2014). In this study, *Holothuria leucospilota*, a species of sea cucumber, was used for biofiltration of wastewaters from a shrimp breeding complex in Chabahar city, located in Sistan and Baluchestan province, by the Oman Sea.

2. Materials and methods

This research was conducted in shrimp Gwater (Gwadar) breeding complex in Chabahar city located in Sistan and Baluchestan province, by the Oman Sea. This farm consists of northern and southern parts. There are 107 farms in the northern part of the shrimp breeding complex, each one being 20 hectares in size and there are also six 200 hectare-farms in the southern part. The study was carried out in April, 2018.

This farming site produces large amount of wastewater, containing different types of pollutants, which enter coastal regions and the Oman Sea. Four sampling stations, including a station for sea water sampling and a station for a control sample were chosen. Station 1 was the control station, station 2 was chosen at the point of where water samples enters the pool, station 3 was where outlet wastewater from the pool flow out and station 4 was chosen just before the wastewater poured into the sea. Three replicate samples were collected from each station and transported to the laboratory in a plastic drum for analyses. In the laboratory, different parameters including pH, dissolved oxygen (DO), nitrate, phosphate, electrical conductivity, turbidity and total dissolved solids (TDS) were measured.

To investigate the biofiltration process, three ponds, comprising of a sedimentation pond, a biological pond, and an aeration pond, were prepared after the main pond. The sea cucumbers, *Holothuria leucospilota,* were collected from the Oman Sea and the Persian Gulf coasts and transported to the experimental pools. The 200 individuals of *H. leucospilota* were placed on the bed of the biological ponds for the purpose of purifying the wastewater from the main pond. After preparation of biological pools with *H. leucospilota*, a certain level of wastewater was allowed to enter the ponds to examine the effectiveness of the filtration process. The wastewater from main pond was allowed to enter the sedimentation pond first, then into the biological pond and finally into the aeration pond. Thereby, the wastewater samples were filtrated by *H. leucospilota*. The filtration rate was measured in different time intervals based on the following formula (Jones *et al*., 2001):

$$V_w = V \times \frac{\ln(C_{t0}) - \ln(C_{tn})}{t \times W}$$

where,

V_w = Filtration level (mg/min/g);

C_{t0} = Number of animals in zero time;

C_{tn} = Number of animals at the end of research;

t = time interval;

w = animal mass (gr).

For statistical analysis of results, one-way ANOVA test was used for comparison of parameter concentrations between different stations, filtration rate at different time intervals, and filtration rate for different animal frequencies. Also, Pearson correlation test was used to investigate the correlation between filtration rate and different parameter concentrations. Both tests were performed at significant level of 0.05.

3. Results and Discussion

Table 1 shows concentration of quality parameters in sampling stations. The pH level was between 8.1-8.3, the highest value was detected in station 3. The dissolved oxygen (DO) value ranged between 4-12 mg/l, the highest and lowest values were detected in stations 1 and 3, respectively. The range of values for measured parameters, in all stations, were from 38.23 to 39.95 µs/cm for EC, 0.54 to 0.68 mg/l for NO_3^-, 16.23 to 22.36 NTU for turbidity, 0.79 to 1.52 mg/l for PO_4^{3-} and 2.09 to 20.35 mg/l for TDS, respectively.

Comparison of quality parameter values between all sampling stations in the shrimp farm is shown in Figure 1. There was significant difference in dissolved oxygen, turbidity, NO_3^-, PO_4^{3-} and TDS levels ($P<0.05$), between all stations, the highest

Table 1. Concentrations of quality parameters in sampling stations at shrimp Gwater (Gwadar) breeding complex

Sampling station	Quality parameter						
	pH	DO mg/l	EC µs/cm	NO_3^- mg/l	Turbidity NTU	PO_4^{3-} mg/l	TDS mg/l
Station 1	8.1	12	38.23	0.54	16.23	0.79	2.09
Station 2	8.1	10	38.45	0.57	17.56	0.94	2.11
Station 3	8.3	4	39.95	0.68	22.36	1.52	20.35
Station 4	8.2	4	39.44	0.56	20.25	1.25	19.89

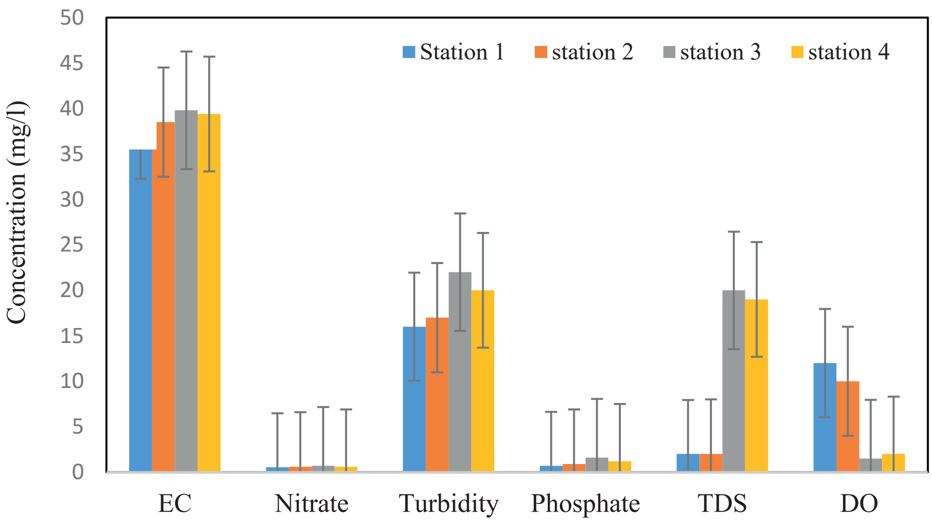

Figure 1. Comparison of quality parameters values between all sampling stations

value for all parameters were detected in station 3, except for dissolved oxygen levels for which the highest value was detected in station 1 and the lowest value in station 3, respectively. The results indicated that station 3 had the highest pollutant content, being the point at the shrimp farm where the wastewater finally leaves the ponds and heads towards the sea.

During the growth period of shrimps, different nutrients specifically nitrate and phosphate fertilizers, different types of growth hormones and other supplements were added to compound feed. Therefore, pollutant concentrations such as turbidity, NO_3^-, PO_4^{3-} and TDS was increased in the shrimp pond, and the outlet wastewater from pond was found to have high levels of quality parameters. Subsequently, pollutant concentrations in the outlet wastewater from shrimp ponds were higher than WHO and FAO standards. The order of quality parameter concentrations in different stations was found to be as follows: station 3 > station 4 > station 2 > station 1.

Wang *et al*. (2007) also showed that pollutant concentrations were high in the wastewater of shrimp ponds, and the levels were higher than environment standards. Moreover, Qing *et al*. (2013) found similar results to the present study, and showed nitrate, phosphate, BOD, salinity and heavy metal concentrations at much higher levels in the shrimp ponds' wastewater compared with the requirements of environmental management standards.

In this study, *H. leucospilota* had high capacity for absorption and removal of pollutants from shrimp farm wastewater. Therefore, *H. leucospilota* is an important species for bio-filtration and improvement of wastewater quality of shrimp and fish farms.

The filtration rate between different times and animal frequencies (densities) were also investigated (Figure 2). The results showed that there were significant differences in filtration rates at different periods ($P<0.05$), the highest and lowest values being on the 8th and first days, respectively, indicated a positive correlation between filtration rate and filtration time. The filtration rate increased with enhancing the filtration time. Over the course of time, the sea cucumbers filtered more wastewater; thereby

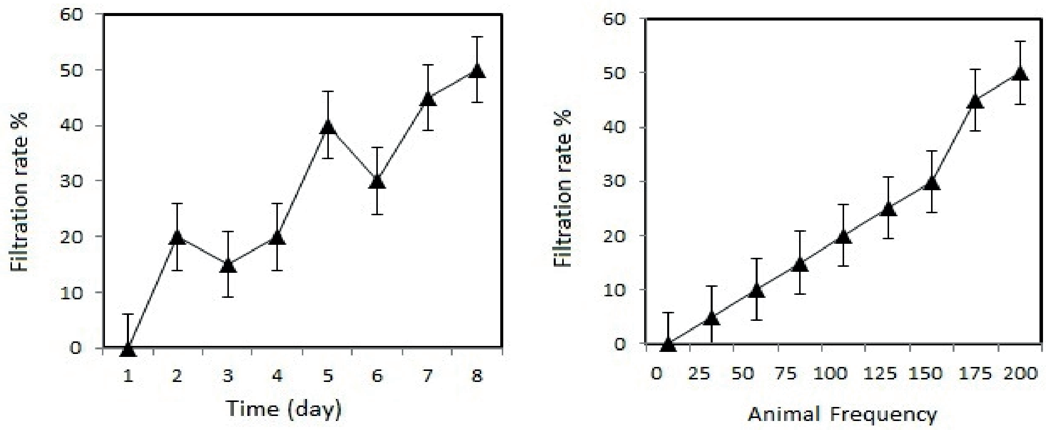

Figure 2. Comparison of filtration rate between different times (Chabahar Gulf, 2016)

the filtration rate was higher in the last few days compared with the first days. Helms *et al*. (2008) had shown similar results with the present study's results that the filtration level was enhanced with increasing the filtration time. Nicolas *et al*. (2016) also reported that there were significant differences in filtration rates during a six-day period and the highest and lowest filtration rates were observed in the sixth and first days, respectively (Nicolas *et al*., 2016). Other studies have also shown positive correlation between filtration rate and filtration period time (Quang, 2014).

Furthermore, there were significant differences in filtration rate between diverse densities of sea cucumber ($P<0.05$), and highest filtration rate was observed at high density (200 individuals of sea cucumbers) (Figure 2).

There was a positive correlation between filtration rate and animal density (Figure 3), so that the higher the number of animals, the larger the amount of wastewater filtrated. The highest and lowest values of filtration rates obtained when the quantities of sea cucumbers were 200 and 25 individuals of *H. leucospilota*, respectively. Yokoyama (2015) showed that the highest value of biofiltration of wastewater was observed in the 250 density of sea cucumber *Apostichopus japonicas*. Brothers *et al*. (2015) reported that there was a positive correlation between filtration value and density of sea cucumber *Parastichopus californicus*.

Pearson Correlation between quality parameters and filtration rate was investigated. Based on the results, there was negative correlation between quality parameters and filtration rate, except for dissolved oxygen (DO). The Pearson correlations (R) for NO_3^-, PO_4^{3-}, turbidity, TDS, EC, and DO were calculated to be 0.944, 0.916, 0.609, 0.781, 0.835, and 0.899, respectively.

Increase in filtration rate reduced the levels of the parameters (NO_3^-, PO_4^{3-}, turbidity, TDS, and EC), which was desirable. However, dissolved oxygen (DO) is a positive parameter that should be increased in concentration for positive effects on the environment and organisms that was noticed in the filtration process and the DO concentration was increased.

The quality parameters before and after filtration in the wastewater were analyzed. Results showed that there were significant differences in parameter concentrations before and after filtration ($P<0.05$), and parameter concentration was higher before filtration compared with after filtration (Figure 4). This meant that parameter concentrations were

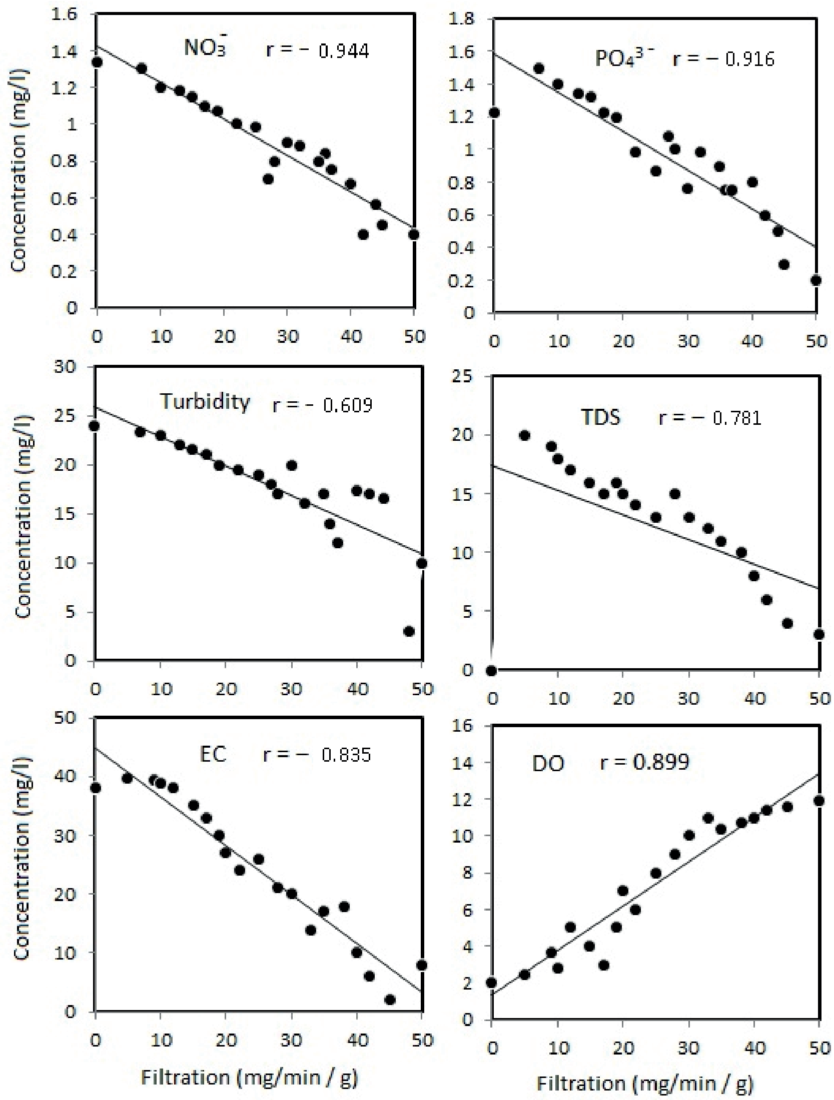

Figure 3. Pearson Correlation between quality parameters with filtration rate

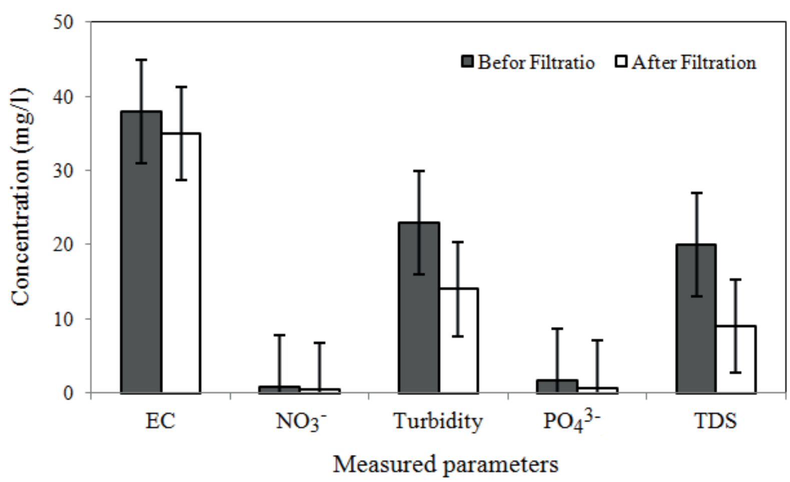

Figure 4. Comparison of quality parameter before and after filtration

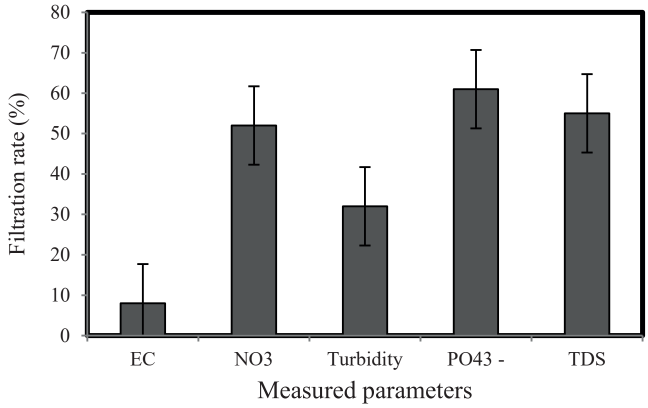

Figure 5. Filtration rate (%) of different parameters by sea cucumber, *H. leucospilota*

reduced by sea cucumber, *H. leucospilota*. This species was able to absorb high concentrations of pollutants from wastewater and sediments, and finally remove pollutants from the wastewater, therefore, all parameters were decreased after filtration compare to before filtration. Brothers *et al*. (2015) showed that sea cucumber *Parastichopus californicus* has high ability for filtration of pollutants from wastewater of shrimp ponds, and pollutant concentrations reduced after filtration in comparsion with before filtration. Moreover, Yokoyama (2015) reported that in the wastewater containing sea cucumber *Apostichopus japonicas*, the pollutant concentrations after filtration decreased significantly compared to before filtration (Brothers *et al*., 2015). It can be concluded that pollutant concentrations in the shrimp pond wastewater were lower after filtration compared to the beginning of the experiment, because *H. leucospilota* has the ability to absorb and remove pollutants.

Figure 5 shows the analysis of filtration rate (%) of different parameters in shrimp pond wastewaters by sea cucumber, *H. leucospilota*. Based on the results, filtration rates were 8%, 52.5%, 32%, 61.8%, and 55% for EC, NO_3^-, turbidity, PO_4^{3-}, and TDS, respectively. The highest and lowest filtration values were for PO_4^{3-} and EC, respectively. The order of filtration values for all parameters were as follows; PO_4^{3-} > TDS > NO_3^- > turbidity > EC. Wang *et al*. (2007) showed that sea cucumber *Apostichopus japonicas* has high ability for removing the pollutants from wastewater of shrimp ponds, and filtration rate were 54%, 37.5%, 11%, 5% and 56% for NO_3^-, PO_4^{3-}, EC, TDS, and turbidity, respectively. Yokoyama (2015) reported filtration rates for NO_3^-, PO_4^{3-}, and TDS by sea cucumber *Apostichopus japonicas* were 63.8 % 58 % and 44.5 %, respectively. Also, Brothers *et al*. (2015) showed that sea cucumber *Parastichopus californicus* has ability for filtration of shrimp ponds wastewater and that filtration rates were 67%, 46%, and 50.5% for Ni, Cd and Pb. Also, they showed that filtration rates for quality parameters were 45%, 5.5%, 45.5%, and 61% for TDS, turbidity, NO_3^- and PO_4^{3-}, respectively.

Conclusion

In this study, sea cucumber, *H. leucospilota* was used for filtration in shrimp farm wastewater from Chabahar Gulf, south of Iran. Some factors such as pH, DO, turbidity, NO_3^-, PO_4^{3-}, EC, and TDS were evaluated in four sampling stations from input water and output wastewaters. The output wastewater of shrimp farms had high concentration of pollutants that needed to be filtered. The biofiltration by *H. leucospilota* was investigated in different periods of time and also with different density of animals. The results showed that *H. leucospilota* had high ability for filtration of wastewater of shrimp farms and filtration rates varied for different time intervals and animal densities. The filtration rate was increased for longer time period and higher density. Finally, it can be said that *H. leucospilota* is a bioindicator for filtration of shrimp farms wastewater.

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