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Filtration conditions for the removal of organic matter in eutrophic waters by freshwater mussels using response surface methodology

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Abstract

In this study, we applied a central composite design to estimate independent variables and establish optimal conditions of filtration rate and feces production that enhance filtration of suspended organic matter by the freshwater mussels *Sinanodonta woodiana*. The results indicated that statistical design methodology offers an efficient and feasible approach for high filtration and low feces production condition optimization. The proposed model equation takes into account the quantitative effect of variables and also the influence of interactions among variables on mussel filtration rate. Under the optimal experimental conditions (mussel size, 13.0 ± 0.2 cm; water current, 17.5 L/h), the experimental filtration rate of 4.47 ± 1.82 L/mussel/h showed a degree of correspondence with the predicted value of 8.4 L/mussel/h, which verified the practicability of this optimum strategy.

Keywords : current, filtration rate, freshwater bivalve, *mussel size, response surface methodology, Sinanodonta woodiana*

1. Introduction

Bivalve mollusks often comprise the highest biomass in the benthos of freshwater and marine ecosystems (Newell *et al*. 2004). Their filter-feeding activity removes phytoplankton and other suspended matter from the water column, both through ingestion and sedimentation of particles in feces and pseudofeces (PF). The ecosystem engineering achieved through bivalve filtration can result in improved light penetration within the water, thus, facilitating the growth of bottom-rooting macrophytes, which in turn can provide habitats for other biota (Fanslow *et al*. 1995; Davenport *et al*. 2000). Indeed, studies in freshwater systems have shown that greater biological richness is associated with greater abundances of unionid mussels, both between different river systems (Aldridge *et al*. 2007) and even within the same lake (Chowdhury *et al*. 2016).

The potential for harnessing the filtration capacity of freshwater bivalves as biofilters has been recognized with regards to the treatment of drinking water (Lammens *et al*. 2004; McLaughlan & Aldridge 2013). In The Netherlands, introduced zebra mussels (*Dreissena polymorpha*) were found to stabilize a phosphorus-enriched lake with clear water (Secchi depth > 1 m) for long periods (Ibelings *et al*. 2007). Zebra mussels have been reported to filter a wide range of plankton, from bacterioplankton to zooplankton, at a rate of approximately 1 L/mussel/day^{-} and have also been reported to improve water clarity (Elliot *et al*. 2008). The filtration rates of suspension feeders that play an important role in benthic and pelagic coupling by filtering material in the water column vary based on many factors, including species, individual size, water velocity, and water temperatures (Comeau *et al*. 2008).

Selecting the conditions that can provide the optimal clearance of suspended material by bivalves is an important step in developing effective biofiltration systems. Response Surface

Methodology (RSM) is a statistical technique that can be used for designing experiments, building models, evaluating the effects of several factors, and searching for optimum conditions for desirable responses (Jeong *et al*. 2014). Using RSM, the interactions and relative importance of different parameters can be evaluated using a limited number of planned experiments (Wang *et al*. 2007).

The main objective of this study was to identify the optimal conditions under which particulate material is removed from the water column by the freshwater mussel *Sinanodonta woodiana* Lea. *S. woodiana* is a large mussel species that is native to the Amur and Yangtze river basins. The species is relatively tolerant of poor water quality and has spread throughout much of southeast Asia and South America as a consequence of fish farming (Kim *et al*. 2009). In this study, we investigated the relative importance of shell size, rate of water flow, filtration rate (FR), and production of feces/PF using RSM.

2. Materials and methods

2.1 Animal collection and experimental design

S. woodiana specimens were collected directly from waterways and streams associated with the Geum and Mankyoung rivers in Korea and acclimated in laboratory aquaria for at least 3 months. The experimental equipment used is described in detail by Lee *et al*. (2009). To study filtration, we used treatment baths of stainless steel (80 × 80 × 145 cm) with a working volume 500 L. Thirty individuals of test mussels were acclimated in holding aquaria for 18 days prior to the commencement of experiments. The acclimation and experimental conditions for filtration by *S. woodiana* were a water temperature of 19 ± 3°C, water flow rate of 24 to 48 L/h, and photoperiod of 12 D:12 L.

2.2 Measurement of filtration rate and production of bivalve feces and pseudofeces

The ash-free dry mass (AFDM) of each mussel used in this experiment was measured according to the method of Hwang *et al*. (2004). After separating the whole body of the mussel from the shell and weighing, it was transferred to a heat-resistant vessel, desiccated at 100°C for 20 min in a drying oven to a constant mass, and then burned in a muffle furnace at 500°C for 2 h (APHA 1995). The AFDM of the mussel body was calculated from the difference in dry weight before and after burning. The filtration rates of the mussels (FR: L/mussel/h) in each experiment were determined using the following equation (Coughlan 1969):

$$FR = V/M \times \ln(T/C)/t,$$

where V is the volume of the experimental reactor (L); M is the total AFDM of the mussels; T and C are the concentrations of suspended solids in water passed through the reactor with and without mussels, respectively; and t (hours) is the duration of the experiment.

The production of feces and pseudofeces by mussels was measured simultaneously by collecting sediments from mussels at 3-day intervals for 9 days. The sedimented particulate matter was harvested in treated baths and placed in sterilized dishes, and the weight of the pellet after drying at 70°C for 1 h was measured. The pseudofeces production of mussels was calculated by the difference in the dry weights (mg/g AFDM/h) of the sedimented particulate matter in the reactor treatments with and without mussels as follows:

$$PFs = V/M \times \ln(T/C)/t,$$

where V is the volume of the experimental chamber (L); M is the total AFDM of the mussels; T and C are the total dry weights of the sedimented particulate matter in the reactor with and without mussels, respectively; and t is the duration of the experiment (hours). Water flow in the chamber was adjusted to 24 L/h and 48 L/h using a water pump.

2.3 Experimental design and the modeling of filtration by mussels

The experimental design for modeling of mussel filtration condition related to body size aimed to determine the optimal levels of three variables, namely, mussel size (x_1), experimental time (x_2), and water flow (x_3) on filtration rates and production of feces. Each factor in the design was studied at three variable (Table 1). For a 2^3 central composite design (CCD) with three factors, including six center points, a set of 30 experiments was carried out. All the variables were taken at a central coded value considered as zero. The minimum and maximum ranges of variables investigated and the full experimental plan with respect to their values in actual and coded form are listed in Table 1. Upon completion of experiments, the average maximum filtration rate was taken as the dependent variable or response (Y). A second-order polynomial equation was then fitted to the data using the multiple regression procedure. This resulted in an empirical model that related the response measured to the independent variables of the experiment. For a three-factor system, the model equation is as follows:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_{23} + \beta_{11} x_{12} + \beta_{22} x_{22} + \beta_{33} x_{32} + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3,$$

where Y is the predicted response; β_0 is the intercept; β_1, β_2, and β_3 are linear coefficients; β_{11}, β_{22}, and β_{33} are squared coefficients; and β_{12}, β_{13}, and β_{23} are interaction coefficients. Data were analyzed using the Minitab statistical software package (Minitab Release 14.12.1, Korea).

3. Results and Discussion

3.1 Effect of mussel size, water flow, and retention time on *Sinanodonta* filtration rate

Among the experimental mussel sizes (mean value ± SE: 8.5 ± 1.0 to 11.4 ± 1.8 cm), water flow (12 to 48 L/h) and retention time (1.5 to 22.7 h), the filtration rate ranged from 0.87 ± 0.17 to 4.47 ± 1.82 L/mussel/h (2.67 ± 1.00 L/mussel/h; Table 2). Filtration rate increased with increasing mussel size and with decreasing water flow rate. The larger mussel group (11.4 ± 1.8 cm) had higher filtration rates than the smaller size group (8.5 ± 1.0 cm). Furthermore, filtration rate in the high water current (48 L/h) was reduced relative to that in the lower current (12 L/h).

3.2 Optimization by response surface methodology

The results of CCD experiments for studying the effects of the three independent variables (mussel size, water flow rate, and retention time) on *S. woodiana* filtration rate is shown in Table 3, along with the mean predicted and observed responses. The regression equation obtained after analysis of variance (ANOVA) produced an R^2 value of 0.7625 (a value of $R^2 > 0.75$ indicated the adequacy of the model, P value < 0.05), which ensured a satisfactory adjustment of the quadratic model to the experimental data and indicated that 76% of the variability in the response could be explained by the model. The coefficients of the regression equation were calculated using Minitab and the following regression equation was obtained:

$$Y = 18.214 - 10.211x_1 + 10.105x_2 + 12.542x_3 - 12.458x_1^2 - 8.243x_2^2 - 9.549x_3^2 + 13.263x_1 x_2 + 17.671x_1$$

x_3 -15.842x_2x_3

Three-dimensional response surface curves were then plotted to determine the interaction of the experimental components and the optimum of each component required for maximum filtration rate. The response surfaces shown in figures 1 and 2 show the relative effect of two variables (mussel size and water flow) with varying retention times. The coordinates of the central point within the highest contour levels in each of these figures corresponds to the optimum filtration rate and feces production of the respective components. Figure 1 shows the response surface for the interactive factors, mussel size (x_1) and water flow (x_2), when the retention time (x_3) ranged from 1.0 to 24.0 h.

The maximum filtration rate of mussels under these conditions was predicted to be 8.4 L/mussel/h, corresponding to maximum levels (+1) of mussel size (13.0 ± 0.2 cm) and water flow (17.5 L/h; Fig. 1). However, the curve also indicates that the response varies in response to the velocity of water flow. With an increase in water flow (greater than 30 L/h) and a decrease in retention time, the production of feces by mussels further increased to 11.1 g AFDM/ind./h (Fig. 2). However, the response surface curves did not show curvature. Instead they were flattened, with mussel size having relatively little effect, but with greater feces production under conditions of greater flow (Fig. 2). Figure 3 indicates that a greater amount of feces was produced at a higher flow rate, but that mussel size had little effect on feces production. These results are relatively consistent with the estimates of the filtration rate and particle retention efficiency of *Crassostrea virginica* (Brusca 2003), which can process up to 37 L/h at 24°C and can capture particles as small as 1 µm in size. In contrast, the filtration rate of marine oysters (up to an approximate valve size of 35 mm) has been reported to be 55 L/ind./d (Pietros and Rice 2003). These filtration activities of bivalves demonstrate the difficulty of determining the standard conditions of feeding and excretion. Accordingly, optimization of filtration conditions is the most important factor for the removal of organic matter by freshwater mussels in eutrophic waters. The surface plots are suggestive of a need for a slower water flow and longer retention time to facilitate minimum feces production. On the basis of these results, the model of mussel filtration indicated that the selected water flows and retention times were limiting, and therefore did not result in a significant curved surface in the response surface graph. Thus, a further decrease in water flow, along with an increase in retention time in the system should be implemented for validation. However, because of experimental limitations, simplified water flow, and short retention time interval, the model was validated only with increased mussel body size. In related research (Kim *et al*. 2011), freshwater bivalves with similar body size showed relatively small differences in filtration rate, but in mussels with a limited range of size and density, the filtration rate would further increase with increasing water temperature. We consider that a multifactorial analytical approach, which takes into account the interaction of independent variables (including individual body size, environmental factors, and experimental conditions) provides a basis for models designed to assess the nonlinear nature of the response under limited experimental conditions.

With regards to the aforementioned results, Ismail *et al.* (2014) explained that since bivalves are an important food source in the aquatic food web, the kinetic data of filter feeding by bivalves could also be utilized in their research designed to elucidate trophic transfer and the biomagnification of organic matters in an aquatic system. These authors stated that the use of environmentally relevant concentrations and treated wastewater can provide the first indication of the potential efficacy of bivalves in removal of contaminants of emerging concern to improve water quality. We believe that additional studies are needed to determine the concentration dependence of organic matter filtration and correlations with bivalve species, age, and prey competition. Previously reported models relating to the reduction of eutrophication through the use of bivalves in a lake system can provide condition but cannot be applied directly in situ to organic matter removal, since the rates of algae and organic matter removal have not been correlated with mussel filtration rates. Results from algal-based studies have confirmed the high filtration efficiency and use of bivalves for improvement of water quality on a large scale, thereby indicating the possible utility of bivalves for improvement of water quality in engineered systems, or as part of ecological rehabilitation (Gifford *et al*. 2007; McLaughlan and Aldridge 2013). These authors have proposed that the conditions or variables that should be taken into consideration when assessing the application of bivalves for water environment improvement are the selection and maintenance of an appropriate bivalve species and population and the optimization of bivalve filtration rates and feces production.

4. Conclusions

(1) The model constructed in the present study indicated that the selected factors of mussel size and water current were limiting, and thus did not result in an adequate surface curvature in the response surface graph. Therefore, a further range of water velocities, along with an increase in retention time, should be assessed for validation purposes. Accordingly, owing to experimental limitations, the model could only be validated with mussel size in the present study.

(2) A central composite design was adopted to screen the key factors and identify optimal conditions for filtration rates and feces production that enhance the filtering of suspended organic matter in water by *Sinanodonta woodiana*. The results indicated

that statistical design methodology offers an efficient and feasible approach for optimizing the conditions that promote high filtration and low feces production.

(3) The proposed model equation illustrated the quantitative effect of variables, and also the interactions among the variables with respect to mussel filtration rate. Under the optimal experimental conditions (mussel size, 13.0 ± 0.2 cm; water currency, 17.5 L/h), the experimental filtration rate of 4.47 ± 1.82 L/mussel/h showed a degree of correspondence with the predicted value of 8.4 L/mussel/h, which verified the practicability of this optimization strategy.

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Conflicts of Interest: The authors declare no conflict of interest

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Table 1. Experimental range and levels of the three independent variables used in response surface methodology in terms of actual and coded factors

Variables	Range of levels					
	Actual	Coded	Actual	Coded	Actual	Coded
Mussel size (cm)	5.0	-1	8.0	0	12.0	+1
Water flow (L/h)	12.0	-1	24.0	0	48.0	+1
Filtration rate (L/mussel/h)	0.5	-1	1.0	0	2.0	+1

Table 2. Filtration rate of the mussel *Sinanodonta woodiana* according to differences in water current and retention time

	Shell length (cm)	Flow rate (L/h)	Retention Time (h)	Filtration Rate (L/mussel/h)
Sinanodonta woodiana	8.5 ± 1.0	12	1.5	0.87 ± 0.17
Sinanodonta woodiana	11.4 ± 1.8	24	22.7	4.47 ± 1.82
Sinanodonta woodiana	11.4 ± 1.3	24	3.9	1.23 ± 0.20
Sinanodonta woodiana	10.6 ± 1.9	24	20.8	3.30 ± 1.03
Sinanodonta woodiana	10.8 ± 1.9	48	10.4	2.30 ± 0.97

Table 3. Experimental designs used in response surface methodology using three independent variables with the center point showing measured and predicted values of *Sinanodonta woodiana* filtration rate

Run order	Shell size (x_1)	Flow rate (x_2)	Production of feces (x_3)	Mean measured response	Predicted response
1	-1	-1	-1	2.36	3.18
2	-1	-1	1	3.40	4.59
3	-1	-1	-1	3.57	4.81
4	-1	-1	1	2.75	3.71
5	-1	1	-1	2.77	3.73
6	-1	1	1	4.06	5.48
7	-1	1	-1	3.21	4.32
8	-1	1	1	1.72	2.33
9	1	-1	-1	2.58	3.47
10	1	-1	1	2.62	3.53
11	1	-1	-1	6.13	8.27
12	1	-1	1	4.78	6.45
13	1	1	-1	2.81	3.79
14	1	1	1	2.47	3.33
15	1	1	-1	13.45	18.14
16	1	1	1	10.71	14.45
17	1	0	0	1.69	2.28
18	1	0	0	6.15	8.29
19	0	-1	0	1.43	1.93
20	0	1	0	4.78	6.45
21	0	0	0	3.97	5.35
22	0	0	0	2.38	3.21
23	0	0	-1	6.53	8.80
24	0	0	1	6.09	8.21
25	0	0	0	6.11	8.24
26	0	0	0	6.45	8.70
27	0	0	0	6.55	8.83
28	0	0	0	6.53	8.80
29	0	0	0	6.28	8.48
30	0	0	0	6.08	8.20