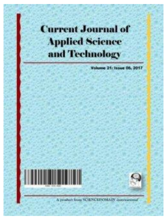

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Influence of Seaweed Extract in Enhancing Phytoremediation of Bio-Digester Effluent by Duckweed

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Authors' contributions

This work was carried out in collaboration between all authors. Author MNK designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors HMS and JKM managed the analyses of the study. Authors JKM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Accumulation of nitrogen and phosphorus in surface water bodies renders aquatic ecosystem non-functional. Duckweed may be used to remove excess nutrients from wastewater, while it requires large surface areas. However, under limited space, nutrient uptake by duckweed may be enhanced by applying seaweed extract (SWE) to the wastewater. The effectiveness of SWE to enhance nutrient uptake under the hot-humid conditions of coastal Kenya has not been assessed. A study was therefore conducted to determine the optimum application rate and a number of applications of SWE to enhance maximum N and P uptake by duckweed from the bio-digester effluent. Four application rates (0, 3, 6, 9, and 12 µL SWE per litre of effluent) and a number of applications of SWE (none, one, two, three and four) were evaluated. A Randomised Complete Block Design with a factorial arrangement of treatment was used. The experiment was replicated for three times. The results showed a significant negative relationships between effluent N content and duckweed biomass (r^2 = 0.982, *P* = 0.013) and effluent P content and duckweed biomass (r^2 = 0.908, *P* =

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0.04). Three applications of SWE reduced the effluent N content by 5.4%. An application rate of 9 µL SWE per litre of effluent reduced the N and P contents of effluent by 9-10% and 20-23% respectively, and increased the duckweed biomass by 22-62%. It is therefore recommended that SWE can be applied at least three times at the rate of 9 µL SWE per litre of effluent.

Keywords: Nitrogen; seaweed extract; duckweed; bio-digester effluent.

1. INTRODUCTION

Human activities such as the use of fertiliser and manure contribute to increased nitrogen and phosphorus in surface and ground water bodies [1-3]. Accumulation of N and P in water bodies results in excessive growth of vegetation that eventually dies and decomposes in the water column. Microorganisms that decompose the vegetative material deplete oxygen in the waterbody, and this may lead to non-functional ecosystems [3]. Increased ammonia-nitrogen in aquatic ecosystem poses a threat to aquatic life such as fish. Preparation of infant formulas using water containing nitrate-nitrogen above 10 mg/L predisposes them to the 'blue-baby syndrome [1]. Nitrates taken in drinking water may be transformed into cancer-causing nitrosamines inside the human body [4]. Environmental remediation is, therefore, necessary to guard against the harmful effects of excessive amounts of N and P in surface and groundwater bodies.

There is an increased interest in using aquatic plants, such as duckweed to remove nutrients from contaminated surface waters and wastewaters [5-6]. Duckweed is superior to other species in nutrient removal since it is easy to harvest and the harvested biomass is fed to livestock as a source of protein. Duckweed belongs to *Lemnaceae* family and has 37 species that are grouped into five genera; *Lemna*, *Landoltia*, *Spirodela*, *Wolffia* and *Wolfiella*. Almost all, *Lemnaceae* species are small free-floating aquatic plants [6-7]. Duckweed is hyper-accumulator [7-8] and has been used in wastewater treatment [9-10]. Most of the nutrient removal by duckweed takes place at the surface water [9]. Therefore, using duckweed to clean wastewater requires large surface area [10]. Land being a limited resource, thus there is a need for the enhancement of nutrient removal by duckweed in wastewater treatment ponds to optimise phytoremediation space by reducing the hydraulic retention time and increasing the amount of wastewater treated per unit area.

Extracts from seaweeds (SWE) contain phytohormones such as cytokinins, auxins gibberellins, and brassinosteroids and has the potential to increase nutrient uptake in plants [11-14]. Seaweed extracts contain a small concentration of potassium (K) but the levels of N and P are very low to be attributed to enhance plant growth [15]. Plant response to SWE application depends on rates, frequency and timing of their application [15]. The effectiveness of SWE to enhance nutrient uptake under the hot-humid conditions of coastal Kenya has not been assessed properly. Therefore, this study aimed at evaluating the effectiveness of Afrikelp (a seaweed extract) in enhancing the potential of duckweed to remove N and P from the bio-digester effluent. The primary objectives were to determine: (i) the amount of P and N extracted by SWE-treated duckweed from bio-digester effluent, and (ii) the optimum application rate and a number of applications of SWE needed to find maximum N and P uptake by duckweed from the bio-digester effluent.

2. MATERIALS AND METHODS

The study was conducted at Pwani University, in Kilifi County, in the coastal region of Kenya. The experimental site was located 30 meters above sea level at latitudes of 3° S and longitudes 40° E. The site receives bimodal rainfall of about 900-1,200 mm per annum and has a mean temperature of 25-30°C. The main season (long rains) is experienced during the months of April-July while the minor season (showers) falls in the months of October-December. The soils are mainly sandy and of low fertility [16].

A South African-based commercial seaweed biostimulants (Afrikelp) was used for the study. A mixture of two duckweed species (*Spirodera* and *Lemna*) was used as the stock material. Such mixtures or polyculture of duckweed species were used because they are known to increase the range of environmental adaptation of duckweed growth [17]. The duckweed stock material was multiplied in 200 L plastic water tanks for a period of three months, after which the experiment was set up in opaque 20 liter plastic containers.

The bio-digester effluent used as a medium for growing duckweed was obtained from a plug-flow tubular plastic that had been charged with cattle manure for biogas production. The effluent was sieved and stored in a 20 litre opaque container and diluted at a ratio of 3:1 (75% water: 25% effluent) using clean tap water. Diluting the effluent with tap water reduced ammonia toxicity on duckweed as observed by Cheng et al. [6].

Two factors were evaluated:

a) SWE application rate and (0, 3, 6, 9, and 12 µL SWE per litre of effluent)
b) Number of SWE applications (0, 1, 2, 3, and 4)

Opaque plastic containers were used to culture duckweed plants. Blocking light using opaque containers reduces the growth of algae that competes with duckweed. The containers measured 45 cm in diameter and 55 cm in depth. Each container had 20 litres of the effluent and 100 g of duckweed fronds (or 628 g/m^2). This was considered as an experimental unit. The duckweed fronds were acclimatised in the effluent media for one week (7 days) before the start of the experiment.

On the first day of the experiment, the duckweed plants were treated with first application of SWE (A1) across all the five application rates of SWE. On the 8^{th} day, the duckweed plants for treatment combinations with two or more applications received the second application of SWE (A2) across all the five application rates of SWE. On the 15^{th} day, the duckweed plants for treatment combinations with three or more applications received the third application of SWE (A3) across all the five application rates of SWE. On the 22^{nd} day, the duckweed plants for treatment combinations with four applications received the fourth application of SWE (A4) across all the five application rates of SWE. The seaweed extract was applied by drenching the effluent below the duckweed fronds using a micropipette. During the experimental period, water lost through evaporation and sample removal from the effluent was replenished by using tap water.

Samples of duckweed tissue (one scoop) and effluent (200 ml) were taken from each container on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day of the experiment. The effluent samples were drawn from below the duckweed mat by using a plastic container. The samples were then kept in a plastic container, and stored in a cooler box and then taken to the laboratory for N and P determination. Duckweed samples for N and P determination were scooped using a strainer (with a diameter of 10 cm), stored in plastic bags and transported in a cooler box to the laboratory on the same day for further analyses.

Nitrogen in the effluent was determined by the Kjeldahl digestion procedure using a Kjeltec 2300 Analyzer model [18]. The major forms of N present in biodigester effluent include organic nitrogen, ammonia, and ammonium (NH_4^+). The anaerobic environment maintains low concentrations of both nitrate (NO_3^-) and nitrite (NO_2^-) in the effluent because oxygen in the nitrate and nitrite is used up by anaerobic bacteria for their respiration process. Phosphorous content in both the effluent and duckweed tissue was analysed by using nitric acid wet digestion method, followed by UV spectrophotometer (Varian, Inc., Palo Alto, Cal. model) according to the Standard Methods for determining phosphate using Ammonium Molybdate Method [18].

A Randomised Complete Block Design (RCBD) was used, with a factorial arrangement of treatments. The treatments were replicated for three times. Data collected were subjected to the analysis of variance (ANOVA) by using the General Linear Model (GLM) procedure of MINITAB 17 [19]. Where F-test showed significant differences at $P \leq 0.05$, the Tukey' test for treatment comparisons was used.

3. RESULTS AND DISCUSSION

3.1 Relationship between Duckweed Biomass and Effluent N and P

The results showed a significant negative relationship (r^2 = -0.982, P = 0.013) between effluent N content and duckweed biomass (Fig. 1). Similarly, there was a significant negative relationship (r^2 = -0.908, P = 0.044) between effluent P content and duckweed biomass (Fig. 1). It was revealed that 98% and 91% of variations in effluent N and P, respectively, could be explained by the linear equation model. The reduction in effluent N and P might be attributed by the result of increased uptake of nitrogen and phosphorus from the effluent by SWE-treated duckweed. This indicates that the capacity of duckweed to remove nitrogen and phosphorus from the effluent was enhanced by SWE through increased duckweed biomass. These findings are in agreement with Fornes et al. [20] who endorsed that hormonal activities in SWE increased the growth of terrestrial plants. In addition, several researchers [15,21,22]] conducted similar kind of experiments and concluded that increased hormonal activity stimulates cell division and elongation resulting to an increase in the nutrient absorptive area of plants roots.

3.2 Effect of SWE Application Rate and Number of Applications on Effluent N and P Contents

The results of this study showed no significant (*P* = 0.05) interaction effect of SWE application rate and number of applications on effluent N content. Similarly, there was no significant interaction effect of SWE application rate and number of applications on effluent P content.

3.3 Effects of Number of Applications of SWE on Effluent N and P Contents

The results showed that number of applications of SWE had significant (*P* =.05) effect on effluent N content by the 14th day after treatment (Table 1) but did not affect beyond this period. There was no significant difference between three and four applications of SWE in their impact on the N content of effluent N. While three applications of SWE reduced the effluent N content by 5.4%, four applications reduced it by 6.8%. This decline in effluent N content with three applications of SWE is probably the result of an increase in duckweed growth and hence an increase in the nutrient absorption area, which in turn increased the absorption of N from the effluent. The chelating effect of SWE as reported by Thirumaran et al. [23] probably released N from the effluent, increasing its availability for uptake by the duckweed. The results showed that number of applications of SWE did not affect effluent P content.

Table 1. Effect of number of applications of SWE on effluent N content on the 14th day after treatment

Number of SWE applications	Effluent N content (g kg^{-1})*
0	0.74 ^{a}
1	0.75 ^{a}
2	0.73 ^{ab}
3	0.70 ^{b}
4	0.69 ^{b}
CV	9.65

** Means followed by the same superscript are not significantly different at P ≤ 0.05*
CV = coefficient of variation

3.4 Effect of SWE Application Rate on Effluent N Content

The result of the study revealed that SWE application rate had a significant (*P* = 0.05) effect on effluent N content by the 21st and 28th day

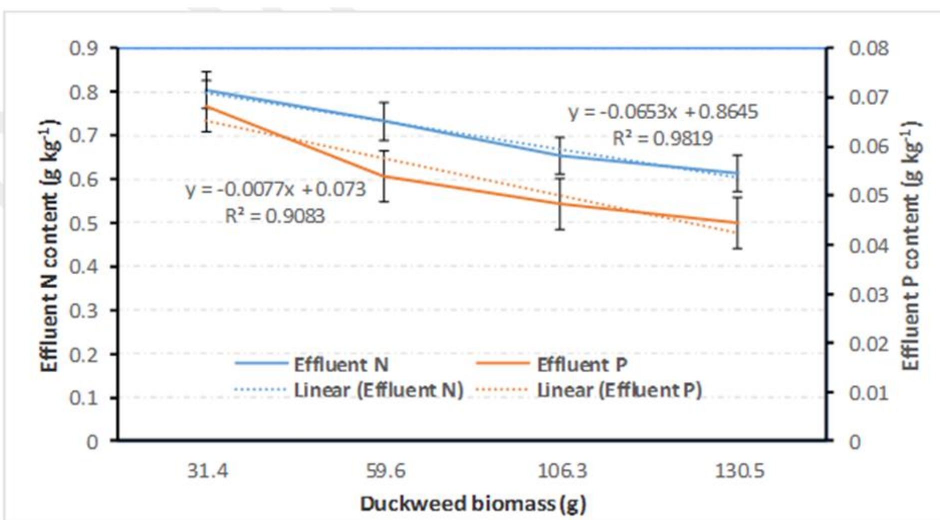

Fig. 1. Relationship between duckweed biomass and effluent N and P content

after treatment (Table 2). An application rate of 6 or 9 µL SWE per litre of effluent reduced the effluent-N content by 7.4-10.3% and 9.1-12.1%, respectively, by the 21st and 28th day after treatment. This shows that SWE applied at this rate (6 µL SWE per litre of effluent) caused a significant removal of N from the effluent. On the 21st day after applying SWE, the rates of 3, 6, 9 and 12 µL SWE per litre of effluent did not differ in their effect on the N content of effluent. By the 28th day after applying SWE, the rates of 6 and 9 µL SWE per litre of effluent did not differ in their effect on the N content of effluent, but the effects of 3, 9 and 12 µL SWE per litre of effluent differed significantly. Comparing biomass accumulation with effluent N, application of 9 µL SWE per litre of effluent resulted in the highest biomass accumulation but the lowest effluent N, while applications of 3 and 12 µL SWE per litre of effluent resulted in reduced biomass but increased the effluent N. This suggests that application of 9 µL SWE per litre of effluent provided the optimal hormones inherent in seaweed that enhanced the highest N uptake by duckweed while 3 and 12 µL SWE per litre of effluent caused less growth and therefore less N uptake. This observation is in agreement with the findings by Fornes et al. [20], who reported that plant hormone has different effects when applied at different application rates.

3.5 Effect of SWE Application Rate on Effluent P Content

The results showed that SWE application rate had significant (P = 0.05) effect on the effluent P content by 21st and 28th day after treatment, respectively (Table 3). On both the 21st and 28th day after treatment, an application rate of 9 µL SWE per litre of effluent significantly reduced the effluent P content by 20% and 23% respectively, as compared to the control. Application rates of 9 and 12 µL SWE per litre of effluent did not differ in their effect on the P content of effluent. This shows that there was no advantage of applying the latter rate of SWE application in terms of effluent P removal. The lower application rates (3 and 6 SWE per litre of effluent) had no significant effect on the effluent P content. This suggests that an application rate of 9 µL SWE per litre of effluent provided the most optimal levels of hormones inherent in the SWE that enhanced duckweed growth and therefore increased the uptake of effluent P by duckweed by the 21st day and 28th day after treatment.

Table 2. Effect of SWE application rates on effluent N content (g kg^{-1}) at 21 and 28 days after treatment

SWE application rate (µL SWE per litre of effluent)	Duration after treatment*	
	21 days	28 days
0	0.68 ^{a}	0.66 ^{a}
3	0.65 ^{ab}	0.63 ^{a}
6	0.63 ^{b}	0.58 ^{bc}
9	0.61 ^{b}	0.60 ^{b}
12	0.62 ^{b}	0.56 ^{c}
CV	10.44	11.22

** Means within a column followed by the same superscript are not significantly different at P ≤ 0.05*
CV = coefficient of variation

It was also found that SWE application rate had significant (P = 0.05) effect on duckweed biomass by the 21st and 28th day after treatment (Table 3). An application rate of 9 µL SWE per litre of effluent increased duckweed biomass by 62% and 22% respectively, as compared with the control. The increased P uptake by duckweed may be attributed to extra demand for P to

Table 3. Effect of SWE application rate on effluent P content and duckweed biomass at the 21st and 28th days after treatment

SWE application rate (µL SWE per litre of effluent)	Effluent P content (g kg^{-1})*		Duckweed biomass (g)*	
	Duration after treatment*		Duration after treatment*	
	21 days	28 days	21 days	28 days
0	0.055 ^{a}	0.048 ^{a}	85.2 ^{b}	128.9 ^{b}
3	0.050 ^{ab}	0.051 ^{a}	100.1 ^{b}	134.9 ^{b}
6	0.048 ^{ab}	0.045 ^{ab}	104.3 ^{b}	137.4 ^{b}
9	0.044 ^{b}	0.037 ^{b}	137.9 ^{a}	157.2 ^{a}
12	0.042 ^{b}	0.039 ^{b}	101.8 ^{b}	140.0 ^{b}
CV	16.72	20.69	24.27	11.44

** Means within a column followed by the same superscript are not significantly different at P ≤ 0.05*
CV = coefficient of variation

sustain increased metabolising energy in nucleotide formation. These observations are in agreement with those reported by Stirk et al. [24] who observed that plant responses to SWE depends on application rates, frequency and the timing of applications in relation to the stage of development of a plant. Earlier studies have also shown that uptake of P by plants growing on effluent depends mainly on their growth rate and availability of P in the effluent [25].

4. CONCLUSION

Effluent-N was significantly reduced by applying SWE at least three or four times. It was also significantly reduced by applying SWE at the rate of 6 or 9 µL SWE per litre of effluent. The application rate of 9 µL SWE per litre of effluent led to the highest duckweed biomass production which coincided with the highest effluent-P removal by the duckweed. This is an indication that the application rate of 9 µL SWE per litre of effluent is effective in enhancing the capacity of duckweed to remove both N and P from the bio-digester effluent. It can, therefore, be recommended that SWE can be applied at least three times at the rate of 9 µL SWE per litre of effluent. Therefore, the use of SWE is an effective and environmentally friendly approach for duckweed-based wastewater treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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