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Potential of *Ulva lactuca* for municipal wastewater bioremediation and fly food

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ABSTRACT

Macroalgae are considered a promising approach for wastewater treatment and could also ultimately provide an alternative animal food source in addition to a biofuel feedstock. Their large size and/or tendency to grow as dense floating mats or substrate-attached turfs lead to lower separation and drying costs than microalgae. In this study, the macroalgae species *Ulva lactuca* (*U. lactuca*) were used to investigate their capacity for treating municipal wastewaters, and the feasibility of using the harvested biomass as a feed for the fruit fly *Drosophila melanogaster*, an animal model for biological research. Results indicated that *U. lactuca* could successfully grow on three types of wastewaters studied with biomass productivities of 8.12–64.3 g·DW (dry weight)/(m^2·d). The secondary wastewater (SW) was demonstrated as the most effective wastewater medium for *U. lactuca* growth. However, both high nitrogen (92.5%–98.9%) and phosphorus (64.5%–88.6%) removal efficiencies were observed in all wastewaters, particularly in primary wastewater and SW, while the highest removal rates (N 24.7 ± 0.97 and P 0.69 ± 0.01 mg/(g·DW·d)) were obtained in centrate wastewater. Moreover, the addition of 20% washed *U. lactuca* into 80% standard fly food (w/w) led to an extended life span and stable body weights in flies while not for the food treatment with 20% unwashed *U. lactuca*. This study demonstrates an effective approach for the macroalgae-based treatment of municipal wastewater and the biomass for animal feed.

Keywords: Wastewater; Macroalgae; Nutrient recovery; *Ulva lactuca*; Animal feed; Flies

1. Introduction

Algae (microalgae and macroalgae) have been considered as biomass feedstocks for the production of third-generation biofuels, as well as the mitigation of greenhouse gas emissions and wastewater treatment [1]. The cultivation of macroalgae does not require fertile land and does not compete with food and agriculture, and can exhibit high biomass productivity (BP) [2]. Moreover, the macroalgal biomass is composed of lipids, carbohydrates and proteins that can be converted to a variety of liquid and solid biofuels (i.e., bioethanol, biobutanol, biodiesel, biocrude) [3–5]. More importantly, macroalgae are larger than microalgae, and they can grow as dense floating mats or substrate-attached turfs [6], thereby potentially offering significant reductions in harvesting and dewatering costs relative to microalgae.

Macroalgae have been primarily employed in two fields related to wastewater bioremediation: nutrient and pollutant removal from municipal wastewaters [7] and removal of toxic metals from industrial wastewater [8]. For example, the macroalgal species *Chaetomorpha linum* (*C. linum*), *Cladophora* sp., *Spirogyra* sp. and *Oedogonium* sp. have demonstrated great capacities for wastewater treatment and biomass production [5,9,10]. However, most studies to date that have examined nutrient removal by macroalgae have focused on wastewaters with low nutrient concentrations [6,11], such as

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fish-farm wastewater with low nutrient concentrations (nitrogen 1.93–2.75 mg/L and phosphate 0.16–0.53 mg/L) [12], aquaculture wastewater (N 4.73–11.34 μmol/L, P 1.2–1.85 μmol/L) [13] and other surface water bodies contaminated by agricultural and stormwater runoff. Studies involving nutrient-rich wastewater treatment using macroalgae have indicated that macroalgae could also have the potential to treat wastewaters with high nutrient concentrations [10,14]. This requires further investigation to expand and demonstrate the application of macroalgae in the bioremediation of municipal wastewaters with high nutrient concentrations and with the potential simultaneous production of macroalgal biofuel feedstocks. In addition, the composition of the macroalgal biomass produced could be affected by the wastewater medium, which could influence subsequent biomass applications.

Macroalgae could be used as feedstocks for a variety of biomass applications, such as fertilizers and soil conditioners [15], biofuels [16], and human and animal food [17]. With high levels of minerals, vitamins, proteins, carbohydrates and polyunsaturated fatty acid but low lipid content, macroalgae have been used as ingredients in food preparations across the world [18]. Macroalgae are not only a food source for marine animals such as the shore crab, sea bass, snakehead and shrimp [19,20] but have also been used as a provider of antibacterial agents for poultry and swine [21,22]. However, some researchers also reported that incorporating macroalgae into the diets of chickens and ducks might have detrimental effects on their growth [23,24]. Hence, the effects of macroalgal biomass on animals should be studied on a case-by-case basis. When using macroalgae as an alternative to traditional animal food, several parameters must be considered (e.g., dose, pre-treatment, temperature, etc.). In modern biological sciences, fruit flies are widely used as an attractive animal model because of their effectiveness as genetic tools; however, food consumption and food waste present concerns in the fly research community. Macroalgae could be investigated as a substitute for standard fly food to improve its economic and environmental viabilities; however, to the authors' knowledge, no such studies have been reported to date.

The purpose of this study is to investigate the possibility of using macroalgae *Ulva lactuca* (*U. lactuca*) for phosphorus and nitrogen recovery from municipal wastewater, and to provide a proof of principle that macroalgal cultivation could be considered as a technology for wastewater treatment and downstream biomass production for animal food. The mortality and weight of flies with *U. lactuca* in their diets were examined to investigate whether the macroalgal biomass could be used as a partial alternative to standard fly food.

2. Materials and methods

2.1. Macroalgae and wastewater

U. lactuca was used as the model macroalgal species and was obtained from a local aquarium store. It was inoculated on Walne's medium with a salinity content of 32‰ [25], in a flat-plate aquarium (35 × 40 × 50 cm) in order to allow acclimatization to laboratory conditions. The aquarium was equipped with an Orphek Atlantik Aquarium LED lighting platform, which can provide appropriate light spectra ranging from 380 to 440 nm and 650 to 670 nm for macroalgal growth. Two air pumps (Tetra Whisper, Canada) equipped with membrane filters provided aeration and mixing condition at a rate of around 200 mL/min. *U. lactuca* from the aquariums were used as the inoculum for the following experiments.

The wastewater used for the *U. lactuca* growth was collected from the Ravensview wastewater treatment plant (WWTP), with an average treatment capacity of 95,000 m^3/d, located in Kingston, Canada. Three types of wastewaters were used for the macroalgal growth, including primary wastewater (PW), secondary wastewater (SW) and centrate wastewater (CW) collected from different sampling sites in the WWTP. The wastewater was stored in the laboratory refrigerator at 4°C until use. The composition of the wastewater was summarized in Table 1.

2.2. Experimental setup of macroalgal growth on wastewater

2.2.1. Macroalgal growth on wastewater

Jar test experiments using 250 mL Erlenmeyer flasks were performed in the laboratory. *U. lactuca* was exposed to one of three wastewaters with salinity maintained at around 32‰: (1) PW, (2) SW and (3) a series of CW (3% and 4%, v/v) diluted with deionized (DI) water. DI water alone (salinity 32‰) was used as a control treatment. All wastewaters were sterilized in an autoclave at 120°C for 20 min. Each treatment had six replicates, and each flask contained a working volume of 200 mL and an initial total biomass of approximately 0.30 ± 0.03 g fresh weight (FW) of *U. lactuca*. Aquarium air pumps (Tetra Whisper, Canada) were connected to in-line filters, and air diffusers were used to provide mixing to the cultures. An Orphek Atlantik Aquarium LED lighting platform was used to illuminate all flasks with a 24-h light cycle at temperatures between 24.0°C and 27.5°C. The flask positions were changed daily to provide similar light intensity exposure to each flask. The volumes of the flasks were kept constant over the experimental period with the addition of DI water every day.

The biomass FW was measured every day; first the collected biomass was centrifuged at 10,000 g for 5 min and then weighed using a Denver Instrument SI-234 balance. Water samples were collected and then filtered through a 0.45-μm vacuum filter for NH_4^+–N, NO_3^-–N, NO_2^-–N and total phosphorus (TP) analyses at the beginning and end of each treatment. Three indicators were used to evaluate the wastewater treatment performance as per Eqs. (1)–(3), including

Table 1
Characteristic of the wastewater

Parameters	Composition concentration (mg/L)		
	CW	PW	SW
COD	477 ± 32	154 ± 41	24 ± 3
NH^{4+}–N	648 ± 57	17.5 ± 3.5	0.35 ± 0.05
NO^{3-}–N	0.04 ± 0.003	0.43 ± 0.02	21.3 ± 1.6
TP	24.8 ± 2.2	1.59 ± 0.38	0.11 ± 0.02
pH	8.43 ± 0.37	7.65 ± 0.81	7.19 ± 0.55

nutrient removal efficiencies (RE, %), treatment efficiencies (TE, %/d) and removal rates (RR, mg/(g·DW·L·d)). The BP (g·DW/(m²·d)) was calculated using Eq. (4):

$$\text{RE}\ (\%) = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

$$\text{TE}\ (\%/d) = \frac{C_0 - C_t}{C_0 \cdot d} \times 100\% \quad (2)$$

$$\text{RR}\ (\text{mg}/(\text{gDW} \cdot L \cdot d)) = \frac{C_0 - C_t}{m_t \cdot t} \quad (3)$$

$$\text{BP}\ (gDW/(m^2 \cdot d)) = \frac{m_t - m_0}{(FW/DW) \cdot A \cdot t} \quad (4)$$

where C_0 and C_t are the nutrient concentrations on the first and final day (mg/L); t is the experimental time (d); m_0 and m_t are the biomass weights on Day 0 and t (g); FW/DW is the fresh to dry weight ratio; and A is the area of the flask (m^2).

2.2.2. Biomass production for fly study

Following macroalgae growth experiment, SW was selected to cultivate *U. lactuca* in the aquarium under similar growth conditions to those noted previously. When the biomass increased to more than triple its initial mass, samples were taken and divided into two parts. One part was thoroughly rinsed with DI water to reduce the salt, sand and gravel until a salinity of <0.5‰ was reached in the rinse water (defined as "washed *U. lactuca*"). The other part was roughly rinsed with DI to remove sand and gravel (defined as "unwashed *U. lactuca*"). After cleaning, both biomass samples were dried at 55°C to a constant DW, powdered manually with a pestle and mortar, sifted through a piece of muslin with a pore size about 10 μm and stored for the fly study.

2.3. Experimental setup of fly feeding study

2.3.1. Flies

Parental *Drosophila melanogaster* of wild type Canton-S strain (Bloomington Drosophila Stock Center at Indiana University, USA) were raised in 10 mL plastic vials and allowed to lay eggs on standard medium (0.01% molasses, 8.2% cornmeal, 3.4% killed yeast, 0.94% agar, 0.18% benzoic acid, 0.66% propionic acid) at room temperature 21°C–23°C, 60%–70% humidity. A 12/12 h light/dark cycle was provided using three light bulbs (Philips 13 W compact fluorescent energy saver) with lights on at 7 am and off at 7 pm every day. Male flies were collected within 2 d following eclosion (defined as Day 1) for the following experiments.

2.3.2. Preparation of different fly food sources

The effects of food on the flies were investigated starting on Day 1, in which two different food sources were provided. They involved the mixture of 80% standard fly standard medium (same component as mentioned above) and 20% washed or unwashed *U. lactuca* (w/w). The dose of 20% was selected according to the optimized dose determined for livestock and swine in previous studies [21,22] as well as our preliminary studies. For each type of food source, at least 250 flies were raised in 10 vials (around 25 flies per vial) under the same conditions noted above. Flies were transferred into fresh food vials with the same food composition every 4 d. A control with 250 flies was included in 10 vials with standard fly food alone. Among the 10 vials with each food treatment (including control), 5 were used to monitor the life span, and the other 5 were used to perform the body weight experiment.

2.3.3. Life span experiment

Flies from five replicate vials (25 flies per vial) of each food treatment (including the control) were maintained as long as feasible, and the deaths of flies were recorded on Day 10, 20, 30, 40 and 50. Flies were considered to be dead when neither voluntary movement nor responses to external stimulation could be observed. The survival percentage on each recorded day was the average of the survival percentages from five vials with the same treatment.

2.3.4. Body weight experiment

The remaining five vials for each food treatment (including control) were used to monitor changes in body weight. The average fly body weight from five vials was recorded on Day 1, 10, 20, 30 and 40 using a Denver Instrument SI-234 balance (accuracy 0.0001 g).

2.4. Chemical analysis

Temperature, pH and dissolved oxygen were monitored using a microprocessor meter with corresponding probes (Fisher Scientific™ Accumet™ Excel XL60). NH_4^+–N and NO_3^-–N were analyzed using a Hach spectrophotometer (Method No. 8171). COD was analyzed with a Hach Model DR/2010 spectrophotometer according to Standard Methods [26]. TP was measured using the Hach PhosVer 3 Method No. 8190 with acid-persulfate digestion. TN was measured using the Hach TNT Persulfate Digestion Method No. 10072. Salinity was measured using Hach Pocket Pro⁺ Multi 1.

2.5. Statistical analysis

For all quantifications and graphs, means and standard deviations are given. For the biomass composition study, one-way analysis of variance (ANOVA) with post hoc Turkey's test was performed to compare the difference between different wastewater cultures. For the fly study, the sample size was 5 in all experiments. Comparison of the survival percentage or body weights of washed and unwashed *U. lactuca* treated flies against control flies was performed as one-way ANOVA with post hoc Dunnett's test. All p values presented were two-tailed. Statistical tests were performed with Prism version 5.0 (GraphPad Software, San Diego, CA).

3. Results and discussion

3.1. Bioremediation of wastewater and biomass production of U. lactuca

3.1.1. Biomass production

Fig. 1 shows the growth performance of *U. lactuca* grown on different types of wastewaters and Walne's medium indicated by the FW and biomass productivities, as well as the other water quality parameters. For the wastewater cultures, the highest growth rate during the 6-d growth cycle was obtained in the SW treatment, followed by PW, 4-CW and 3-CW (Fig. 1(a)), indicating that *U. lactuca* could grow well on all types of wastewaters employed although SW appeared to be the most effective growth medium with nitrate as the dominant nitrogen form. When cultivated on SW and PW, *U. lactuca* could achieve biomass productivities as high as 64.3 ± 3.38 and 21.4 ± 0.86 g·DW/(m²·d), respectively (Fig. 1(b)), which was lower than that of *U. lactuca* grown on Walne's medium (87.2 ± 0.52 g·DW/(m²·d)), but comparable with those (22–55 g·TS·DW/(m²·d) and 37.6 ± 8.6 g·DW/(m²·d)) reported in studies involving *U. lactuca* cultivation on natural seawater [27,28]. The lower biomass productivities (7.75–10.4 g·DW/(m²·d)) observed in the CW treatments were still comparable with those of *C. linum* (8.45–11.8 g·DW/(m²·d)) grown on PW, SW and a series of CW as demonstrated in a previous study by Ge and Champagne [10].

The pH was noted increased due to the significant macroalgal biomass production in both SW and PW treatments, whereas in 3- and 4-CW treatments pH was relatively stable compared with initial values, but was found to be higher with values above 8.0 (Fig. 1(c)). Over the experimental period, salinities were relatively constant around 23.5‰–26.3‰ in all treatments, and the temperatures were maintained at 23.9°C–25.7°C (Figs. 1(d) and (e)).

These observations suggest that municipal wastewater could be used as a marine macroalgal growth medium to reduce water and nutrient requirements. However, it should be noted that the raw CW contains high concentrations of nutrient and other constituents such as heavy metals and/or free ammonia that is toxic, lipid soluble, and can traverse biological membranes in its uncharged form under pH 8.5 [29], which could potentially inhibit macroalgal growth. As such, direct or full strength use of raw CW should be avoided as a macroalgal growth medium; corresponding pre-treatments or strategies should be established to alleviate the adverse effects and facilitate macroalgal growth on a case-by-case basis, such as the integration of CW and SW, and supplementation of CO_2 to lower pH during the macroalgal growth process.

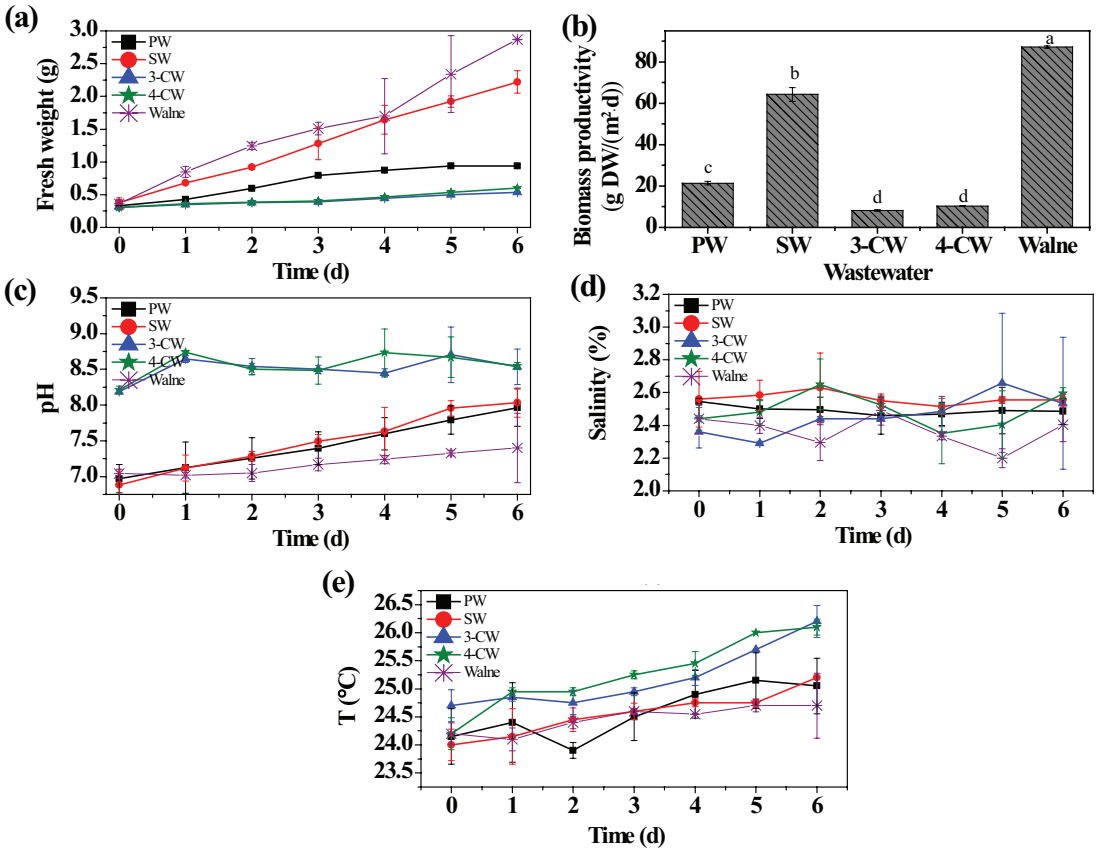

Fig. 1. Comparisons of (a) biomass production (g) by fresh weight, (b) biomass productivity (g·DW/(m²·d)), (c) pH, (d) salinity (‰) and (e) temperature (°C) for batch studies conducted in 250 mL Erlenmeyer flasks where *U. lactuca* were cultivated on PW, SW, 3-CW and 4-CW. Different lowercase letters on the bars indicate significant difference ($p < 0.05$).

3.1.2. Nutrient removal

Satisfactory nitrogen (ammonia or nitrate) removal capacities of *U. lactuca* were observed, with REs between 92.5% ± 1.71% and 98.9% ± 0.23% and TEs at 11.6%–12.4%/d in three types of wastewaters studied, respectively (Table 1), even though different growth dynamics and biomass productions were obtained as noted above. However, the nutrient RR used for evaluating the nutrient removal capacities per gram of biomass per day varied significantly with wastewater type. The RR of nitrogen in 3-CW (24.7 ± 0.97 mg/(g·DW·L·d)) was almost 24-fold greater than that observed in SW (1.09 ± 0.10 mg/(g·DW·L·d)), which warrants further investigation into the nitrogen removal mechanism in macroalgae-based wastewater treatment systems. Compared with nitrogen removal, *U. lactuca* showed lower phosphorus removal capacities particularly in CW. REs and TEs ranged between 64.5%–88.6% and 8.07%–11.1%/d, respectively (Table 2). The RRs of phosphorus exhibited a similar trend to that observed for nitrogen, where higher RRs were observed in CW and PW with a primary nitrogen form as ammonia, compared with SW where nitrate was the primary nitrogen form. These results were consistent with early demonstration that many marine macroalgae generally preferred ammonia over nitrate by up to 50% [30]. In a macroalgal growth system, the nutrient removal/uptake rates were influenced by various factors including physical (light, temperature, etc.), chemical (nitrogen sources and forms, etc.) and biological (nutritional history, life history, type of tissue, interplant variability, etc.) factors. However, it is worth noting that the nutrient removal indicators (RE, TE and RR) calculated in Table 2 included all nutrient losses between the influent and effluent concentrations. For example, denitrification and Anammox, as well as volatilization of ammonia would also likely contribute to nitrogen removal in addition to macroalgal uptake, although wastewater sterilization was performed prior to the experiments. Specific mechanism of alternative pathways for nitrogen assimilation and removal should be further investigated. In addition, apart from the macroalgal metabolic assimilation process, phosphorus removal from wastewaters could be facilitated through struvite precipitation in the presence of phosphorus, ammonium and magnesium, under appropriate pH conditions [31,32]. Therefore, further research is required to more fully understand the nutrient removal mechanism and their interactions.

Similar bioremediation capacities of macroalgae for wastewaters have been reported in literatures. Sode et al. [33] found a maximum nutrient RR of 11.35 mg N/(g·DW·L·d) and 1.35 mg/(g·DW·L·d) for *U. lactuca* treating reject water from anaerobically digested wastewater equivalent to 1.4 N·mg/L. Da Silva Copertino et al. [34] reported mean uptake rates by *Ulva clathrate* of 0.383 g·N/(m^3·d) and 0.099 g·P/(m^3·d) in an investigation using a series of outdoor tanks rectors, receiving wastewater directly from a shrimp aquaculture pond. In a study by Ge and Champagne [10], another macroalgae species *C. linum* also exhibited similar nutrient RRs of 7.34–20.1 mg·N/(g·DW·L·d) and 0.13–0.72 mg·P/(g·DW·L·d) for municipal wastewaters. Therefore, these results suggested that the bioremediation of municipal wastewater using macroalgae such as *U. lactuca* could be possible and allow for simultaneous biomass production and recovery.

3.1.3. Biomass composition

The compositions of carbon, nitrogen and phosphorus in the macroalgal biomass varied between wastewater cultures (Fig. 2). Specifically, significant differences ($p < 0.05$) in carbon content were observed between PW and SW, between PW and 3-CW, as well as between SW and 4-CW cultures. Similarly, nitrogen percentages in biomass were different between PW and SW, between SW and 3-CW, and between SW and 4-CW cultures. The phosphorus in the biomass cultured on 4-CW

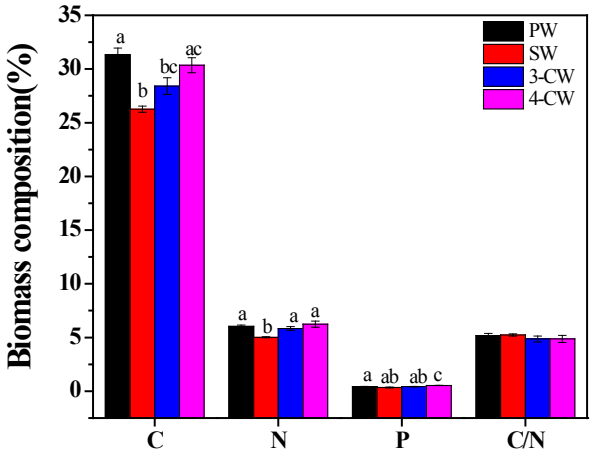

Fig. 2. Biomass composition of carbon (C, %), nitrogen (N, %), phosphorus (P, %) and C/N in biomass after cultured in different wastewaters for four continuous growth cycles (24 d). Different small letters on the bars indicate significant difference ($p < 0.05$).

Table 2
Nutrient removal and treatment efficiencies and removal rates in *U. lactuca* after exposure to different types of wastewaters for 12 d

Wastewater	RE (%)		TE (%/d)		RR (mg/(g·DW·L·d))	
	N	P	N	P	N	P
PW	98.7 ± 0.62	88.6 ± 1.24	12.3 ± 0.08	11.1 ± 0.16	4.51 ± 0.05	0.44 ± 0.01
SW	98.9 ± 0.23	77.7 ± 14.1	12.4 ± 0.03	9.72 ± 1.76	1.09 ± 0.10	0.04 ± 0.01
3-CW	92.5 ± 1.71	64.5 ± 3.92	11.6 ± 0.21	8.07 ± 0.49	24.7 ± 0.97	0.69 ± 0.01
4-CW	98.8 ± 0.34	66.8 ± 4.47	12.4 ± 0.05	8.34 ± 0.56	16.8 ± 0.34	0.38 ± 0.07

Note: The ratio of FW to DW is 3.8.

was significantly different from the biomass cultured on the other three types of wastewaters. Nielsen et al. [35] also reported that the tissue contents of nitrogen and phosphorus varied with the concentrations of liquid pig manure used for *U. lactuca* growth, which influenced the subsequent biomass applications.

Anaerobic digestion of *U. lactuca* to methane was considered more promising compared with other application such as combustion, due to their high ash, alkali and moisture contents [27]. It was demonstrated that the cultivation of *U. lactuca* on wastewaters would improve the environmental and economical sustainability of the *U. lactuca*-based methane production process. However, it should be noted that a C/N ratio of 20–30 has been shown to be more suitable for methanogenic bacteria activity with no inhibition of high pH and ammonia concentrations [36]. In this study, the C/N ratios of the biomass were relatively similar ranging between 4.87 ± 0.27 and 5.24 ± 0.11 regardless of the wastewater culture. These suggested that these considerably lower C/N ratios observed for *U. lactuca* grown on municipal wastewater employed in this study was not well suited for the direct anaerobic digestion as feedstocks. However, the combination of *U. lactuca* with the other carbon-rich biomass such as the waste yeast-fermentation beer from corn-to-beer industry [37] as a co-substrate could be considered to balance the carbon and nitrogen concentrations in an overall co-digestion system.

3.2. Biomass application: food supply for flies used for biological research

A number of physiological parameters could be used to examine the health of common laboratory animal species, such as body weight, organ weight, organ volumes, blood flow speed, respiratory rate and life span [38]. In this study, life span and body weights were the two parameters selected to investigate the fly health and to determine whether the inclusion of macroalgae could be considered as an alternative to standard fly food. 20% of *U. lactuca* was chosen based on our unpublished results, and the optimized dose was added for livestock and swine as reported in previous studies [21,22].

To test the effect of food source treatment with washed and unwashed *U. lactuca*, the survival percentages and body weights were measured on specific days. Survival percentages were noted to vary with food treatment (Fig. 3). The survival percentages of control flies and flies treated with washed *U. lactuca* did not decrease until Day 30, and significantly higher survival percentages were observed on Day 30, 40 and 50 in washed *U. lactuca* treated flies (Day 30, 40 and 50: post hoc Dunnett's test: $p < 0.001$). However, the survival percentage of unwashed *U. lactuca* treated flies started to decrease significantly on Day 20 and was reduced to less than half of the survival percentage of the control flies on Day 50 (Day 20: post hoc Dunnett's test: $p < 0.05$; Day 30, 40 and 50: post hoc Dunnett's test: $p < 0.001$).

The *U. lactuca* treatment also affected the fly body weights. Because several flies had died by Day 40 and the results would be less accurate with fewer flies, the body weights were measured only until Day 40. The initial body weights of the food source treated flies were indistinguishable from the control flies on Day 1 (post hoc Dunnett's test: $p > 0.05$ for washed *U. lactuca*; post hoc Dunnett's test: $p > 0.05$ for unwashed *U. Lactuca*; Fig. 4). The body weights of control flies increased until Day 20 before starting to decrease. The body weights of washed *U. lactuca* treated flies, however, remained at a similar level on all tested dates, and they were significantly larger than control flies on Day 30 and 40 (Day 30 and 40: post hoc Dunnett's test: $p < 0.001$). On the contrary, the body weights of unwashed *U. lactuca* treated flies decreased significantly starting on Day 20 compared with control flies (Day 20 and 40: post hoc Dunnett's test: $p < 0.001$; Day 30: post hoc Dunnett's test: $p < 0.05$).

It has been reported that exclusive consumption of *U. lactuca* could have detrimental effects on blue crab (*Callinectes sapidus*) due to the production of toxic exudates [39] or the insufficient nutrition provided [40]. Also, <10% of the food consumed by blue crabs is *U. lactuca*. The current study demonstrated that 20% washed *U. lactuca* could have positive effects on flies resulting in extended life spans and stable body weights. These findings have implications for animal

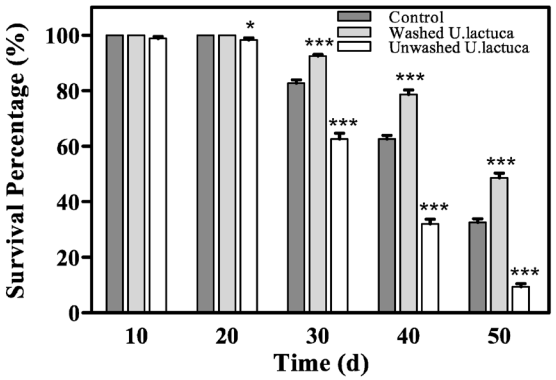

Fig. 3. Survival percentage (%) of flies treated with washed or unwashed *U. lactuca* and control flies on Day 10, 20, 30, 40 and 50. Asterisks (* or ***) indicate $p < 0.05$ or 0.001, respectively, by post hoc Dunnett's test against control flies.

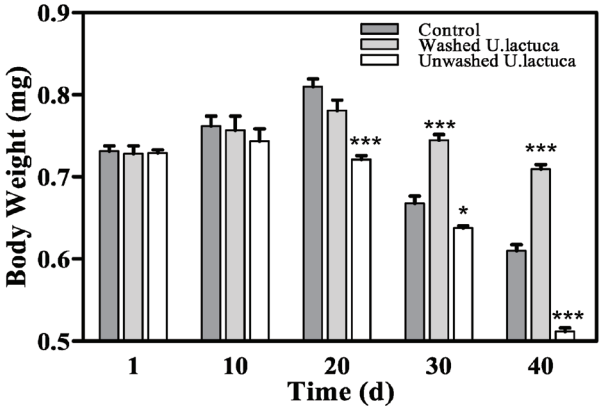

Fig. 4. Body weight (mg) of flies treated with washed or unwashed *U. lactuca* and control flies on Day 1, 10, 20, 30 and 40. Asterisks (* or ***) indicate $p < 0.05$ or 0.001, respectively, by post hoc Dunnett's test against control flies.

laboratories, as fly food with the inclusion of 20% washed *U. lactuca* could become an alternative to the standard food. Similarly, the methanolic extracts of *Chondrus crispus*, a red macroalgae species, have been demonstrated to attenuate oxidative stress and increase the life span in *Caenorhabditis elegans*, probably due to the high amount of bioactive compounds in macroalgae [41]. Although the effect of macroalgae consumption on animal body weight has not been reported to date, to the authors' knowledge, the influence of microalgae has been demonstrated, where it was indicated that the riboflavin and vitamin A in *Chlorella* sp. might be responsible for the improved growth in chicks [42]. In vertebrates including humans, dietary salt is suggested as a major contributing factor to hypertension and some other cardiovascular diseases. The results presented in this study would also suggest that the amount of salt in unwashed *U. lactuca* could also have a substantial impact on survival and body weights, which is consistent with previous studies showing the negative consequence of excess salt intake [43,44]. Moreover, the other algae types, such as red and brown algae, have been shown to improve growth and survival better than *U. lactuca* in sea urchin (*Strongylocentrotus Drobachiensis*) [45], indicating that selection of algal type might also have an effect on fly life span and body weight. Although the survival percentage and body weights were improved by washing of *U. lactuca*, based on the current study, it is still unclear whether it would affect other body functions, such as motor and sensory functions, energy consumption, and nutrition metabolism. Thus, the central and peripheral nervous systems and metabolic pathways of algae-treated animals, including flies, should be examined in future studies. In addition, although it was concluded that the concentrations of the heavy metals (i.e., arsenic, lead, mercury and cadmium) in *U. lactuca* grown on liquid manure were all below the maximum recommended dietary levels and it was relatively safe to include *U. lactuca* in animal food [35], the contents would be variable depending on wastewater used in the cultivation, and therefore, the biomass produced in wastewater cultures would need to be specifically monitored on a site-by-site basis.

4. Conclusion

Effective nitrogen and phosphorus removal was observed for macroalgal cultivation in all three types of wastewaters employed in this study. The findings indicated that the growth of macroalgae may be an effective wastewater remediation technique with the added benefit of being a strong candidate for animal feed. The biomass composition varied with the wastewater type used for macroalgal growth. The survival percentages and body weights of macroalgae-treated flies indicated that washed rather than unwashed 20% *U. lactuca* (w/w) could be applied as a partial substitution of traditional fly food.

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