Supporting Information

for

Cultivation of the marine macroalgae *Chaetomorpha linum* in municipal wastewater for nutrient recovery and biomass production

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S1. Wastewaters

The three different types of wastewaters used for macroalgal growth in this study, included primary wastewater (PW), secondary wastewater (SW) and centrate wastewater (CW). They were collected from Ravensview WWTP in Kingston, Canada.

The PW refers to wastewater collected after treatment in the primary clarifiers, in which the floating materials, grit, grease, fats and oils in the raw wastewater have been removed via primary treatment and in the previous screening processes. The SW was collected from the effluent of the biologically aerated filters (BAF), which includes chlorine disinfection, such that pathogenic microorganisms were largely eliminated from the SW used in this study. The CW was collected from the supernatant of the sludge dewatering process. At the Ravensview WWTP, solids from the raw sewage entering the plant and from the BAF backwash water are settled in the clarifiers and then pumped into the thermophilic digester operated at 55°C, with a retention time of 15 days. The thermophilic anaerobic digestion consumes carbon, produces methane gas and also assists in the destruction of pathogenic organisms in the solids. Next, the solids enter two mesophilic digesters in series operated at 36°C, with a retention time of 15 days in each digester, prior to being stored in the secondary digester and dewatered with a high-speed centrifuge. After the centrifuge process, the liquid or centrate which is high in nutrients and organics, is generally returned to the WWTP and blended with the raw wastewater for treatment.

S2. Macroalgae cultivation system

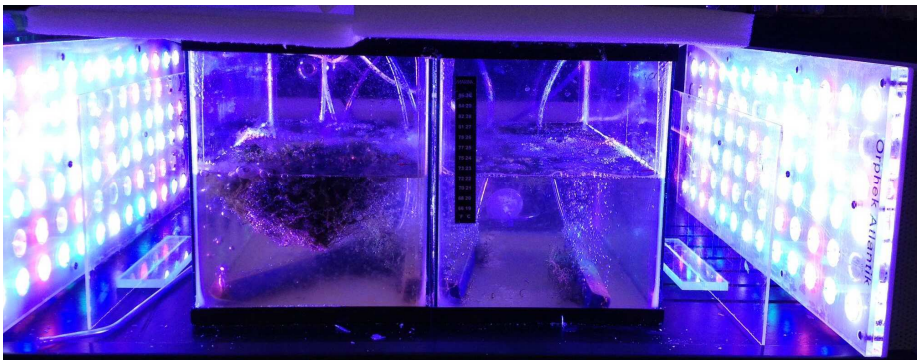

Figure S1. *Chatomorpha linum* (*C. linum*) cultivation system in a flat-plate aquarium (35×40×50 cm), equiped with an Orphek Atlantik Aquarium LED lighting platform.

S2. Extraction and quantification of cell composition analysis

Protein extraction was conducted according to an approach presented by Barbarino et al., [1] and modified as follows: 30 mg of powdered *C. linum* was first immersed in 8 mL of DI water for 12 h, and then the suspension was centrifuged at 4°C, 15,000 g for 20 min. The supernatant was collected and the pellet was re-extracted with 2.0 mL of 0.1 N (or 2 M) NaOH. The mixture of NaOH and pellets were kept at room temperature for 1 h with occasional manual shaking and then centrifuged at 21°C, 15,000 g for 20 min. The supernatants of the NaOH and pellet mixtures were combined with the supernatant of the first fractions and the remaining pellets were discarded. The final volume of the extract was approximately 10.0 mL. Finally, the protein concentration in the collected supernatant was quantified by Bio-Rad DC protein assay (Cat. 500-0111, Bio-Rad Laboratories, Hercules, U.S.) using bovine serum albumin as the standard.[2]

Lipid content was assessed according to an approach presented by Přibyl et al.[3], and modified as follows: a mixture of 0.5 mL of PBS (8 mM Na_2HPO_4, 2 mM NaH_2PO_4, 140 mM NaCl, pH 7.4) and 1 mL of glass beads (diameter 0.5 mm) were added to a glass test tube containing approximately 10 mg powdered *C. linum*. Then the tube was vortexed using a high-speed vortex mixer for 4 min at 3000 rpm, interrupted every 1 min by tap water cooling of the test tubes; 3 mL of extraction solution (methanol/chloroform, 1:2 v/v) was added, samples were shaken briefly with a vortex mixer at 1000 rpm, and lipids were allowed to be extracted overnight at room temperature. To produce a biphasic layer, 1 mL of distilled water was added, and the samples were centrifuged (5,000 g, 10 min, 20 °C). The lower organic phase was then drained using a micropipette, and the extraction procedure was repeated with 2 mL of the extraction solution for an extraction period of 2 h. The collected organic phases were gathered

into a pre-weighed Petri dish, the chloroform was evaporated at 50°C, and the extracted lipids were weighed.

The total carbohydrate was determined by the difference of the protein, lipid, and ash contents in dry biomass without considering the crude fibers. All protein, carbohydrate and lipid contents were determined in 3 replicate measurements of the same sample.

S4. Ash and moisture contents in biomass

Table S1 Ash and moisture contents in biomass cultured in different types of wastewater with different operational strategies

Wastewater	Strategies	Ash content (%, DW)	Moisture (%)
PW	Control	10.6±0.1	17.9±0.5
	SF	10.9±0.1	16.4±0.5
	CO_2	9.5±2.0	16.0±0.1
SW	Control	12.3±1.0	16.4±0.7
	SF	12.6±0.6	14.3±1.5
	CO_2	12.4±1.2	17.2±2.2
PW	Control	10.8±0.2	15.8±1.7
	SF	9.1±0.1	17.8±0.3
	CO_2	10.2±1.5	16.5±1.3

S5. Changes in pH, salinity and temperature

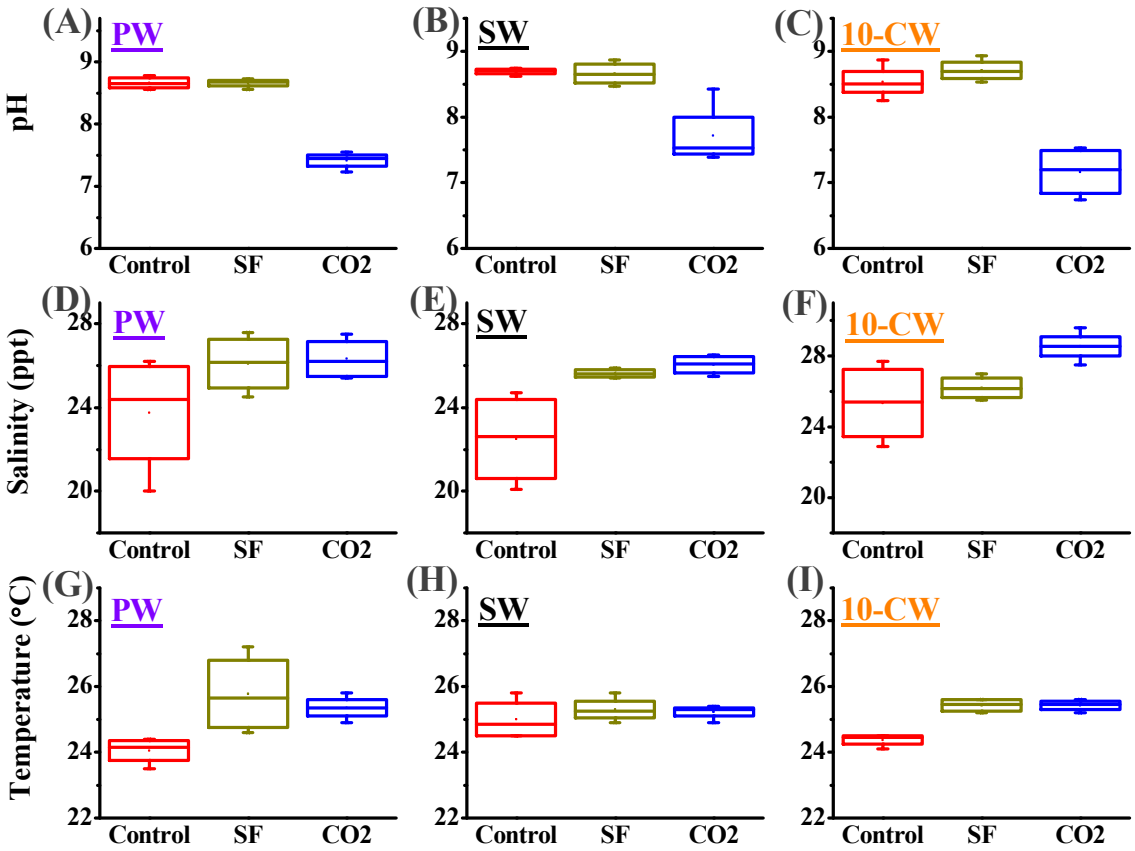

Figure S2. Changes in (A-C) pH, (D-F) salinity (ppt) and (G-I) temperature (°C) in PBRs where *C. lium* was cultivated on primary (PW), secondary (SW), 10 % centrate wastewaters (10-CW) without feeding or CO_2 supplementation strategies (Control), with step feeding (SF) and with CO_2 supplementation strategy. The control PBRs were operated using single feeding and air sparging.

S6. Carbon and nitrogen composition for macroalgae cultured using different strategies

Table S2 Carbon (C, %) and nitrogen (N, %) composition of biomass cultured without feeding or CO_2 supplementation strategy (Control), with step feeding (SF) and with CO_2 supplementation strategy.

Wastewater	C (%)			N (%)		
	Control	SF	CO_2	Control	SF	CO_2
PW	31.11±0.31	30.87±0.63	35.8±0.21	2.83±0.57	2.95±0.26	4.02±0.03
SW	26.5±0.21	27.4±0.18	29.1±0.32	2.47±0.16	2.54±0.43	3.21±0.22
10-CW	39.0±0.47	38.7±0.22	42.3±0.33	6.72±0.33	7.14±0.04	7.89±0.11

S7. Biomass composition of various green macroalgae

Table S3 Composition proportions of proteins, carbohydrates and lipids in green macroalgae reported in the literature.

Macroalgae	Source or Medium	Protein (%,DW)	Carbohydrate (%,DW)	Lipid (%,DW)	Reference
Ulva lactuca	Market	7.0	54.3	6.2	[4]
	Romanian Black Sea	7-30	41-62	1-3	[5]
Ulva pertusa	Market	6.30±0.25	59.07±0.2	2.39±0.1	[6]
Ulva fasciata	Hawaiian Islands	12.3±0.5	20.6±0.7	3.6±0.1	[7]
	Hawaiian Islands	8.8±0.4	17.1±1.3	5.1±0.2	[7]
Enteromorpha intestinalis	Romanian Black Sea	7-20	30-45	1-3	[5]
	Hawaiian Islands	11.4±0.8	22.2±0.6	5.2±0.5	[7]
	Darwin Harbour, Northern Territory, Australia.	3.2	18.7	1.8	[8]
Entermorpha flexuosa	Hawaiian Islands	7.9±0.5	39.9±2.3	5.6±0.2	[7]
Codium reediae	Hawaiian Islands	10.5±0.3	4.5±0.1	5.1±0.5	[7]
	Hawaiian Islands	7.0±0.3	8.2±1.3	6.3±0.1	[7]
Caulerpa lentillifera	Hawaiian Islands	9.7±0.4	11.8±0.8	7.2±0.3	[7]
Caulerpa racemosa	Northern Territory, Australia.	6.8	16.6	3.8	[8]
Halimeda macroloba	Northern Territory, Australia.	6.6	4.7	2.3	[8]
Monostroma oxyspermum	Hawaiian Islands	9.6±0.2	31.8±0.8	3.8±0.1	[7]
Chaetomorpha linum[a]	Monastir, Tunisia	10.56±0.22	42.45±2.94	1.89±0.04	[9]
Chaetomorpha linum[a]	Primary wastewater	7.50±0.31	78.7±0.35	1.74±0.07	This study
	Secondary wastewater	3.31±0.36	80.1±0.72	1.69±0.37	This study
	1% centrate wastewater	6.60±0.78	79.0±0.84	1.44±0.02	This study
	2% centrate wastewater	7.46±1.54	80.2±3.11	1.31±0.16	This study
	10% centrate wastewater	8.65±1.09	77.3±2.54	1.14±0.17	This study
	Commercial medium	5.13±0.85	77.9±0.07	1.42±0.21	This study

a. All compositions reported here is form *C.linum* biomass cultivated in PBRs with single feeding and air sparging.

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