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A Novel System Integrating Electrolysis and Ionic Membranes (EIMs) Enables the Artificial Carbon Concentration and Alleviation of Metal Cation Stress in Microalgae Cultivation

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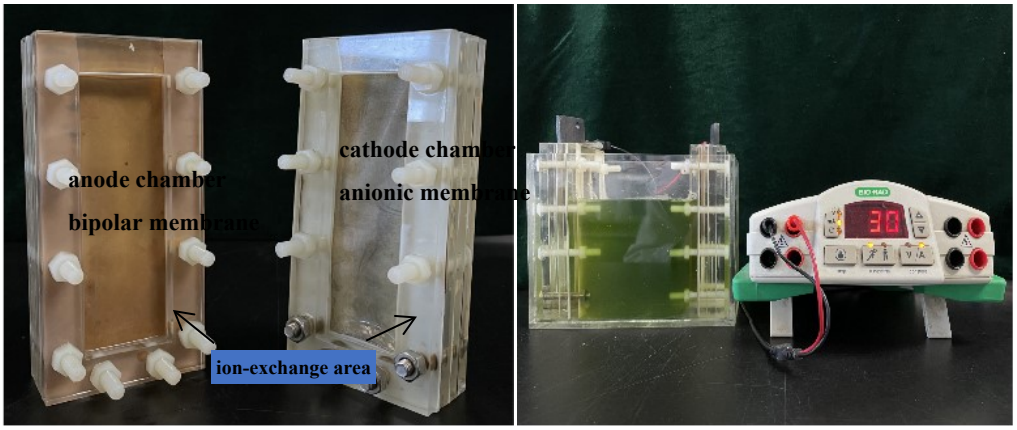
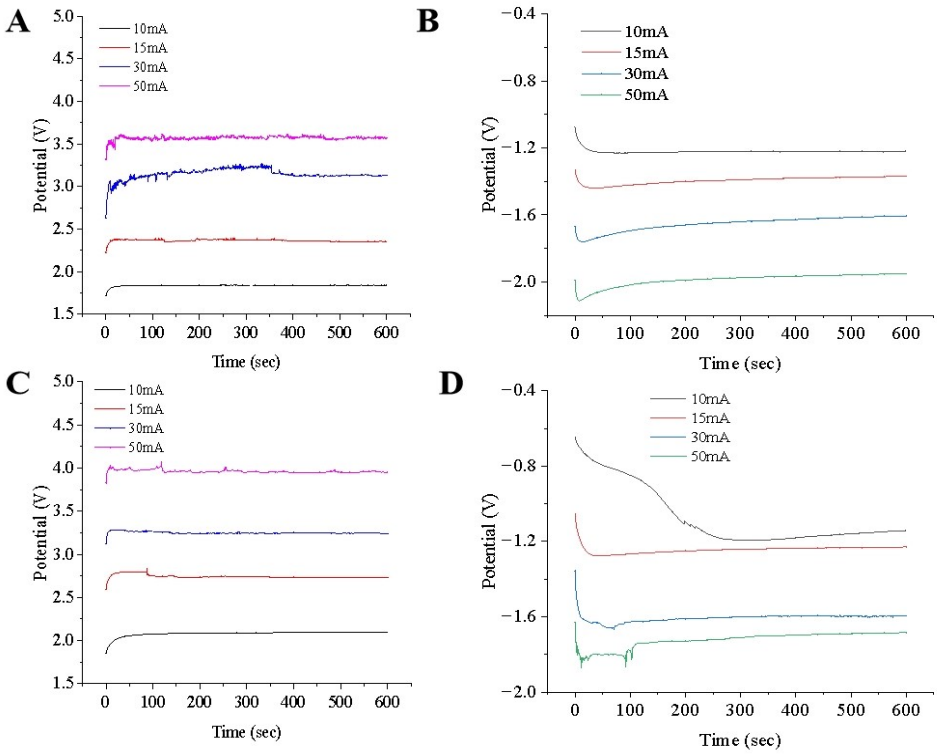
Figure S1-S6

Figure S1. Assembly diagram of the anode and cathode chambers in EIMs.



The EIMs consisted of two parallel cuboids, the andoe and cathode chambers. Each independent chamber (cathode and anode) was assembled with acrylic plates and soft silicone pads with a size of 6.5 (length)×3 (width)×14 (height) cm and an ion-exchange area of 35 cm^2.

Figure S2. The electrode potentials of EMIs added with sea-water or fresh water BG11 medium.

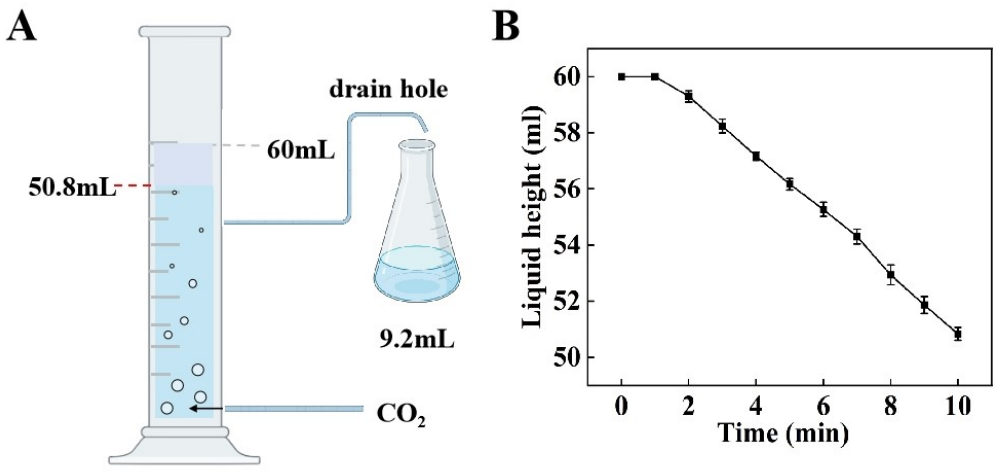


The detected electrode potentials of EIMs when either sea-water or fresh water BG11 medium was used in the system. The electrode potentials were measured by a CHI660E electrochemical workstation at room temperature with an integrated three-electrode system within Ag/AgCl reference electrode. A, anode potential of EIMs when sea-water BG11 medium was used; B, cathode potential of EIMs when sea-water BG11 medium was used; C, anode potential of EIMs when fresh water BG11 medium was used; D, cathode potential of EIMs when fresh water BG11 medium was used.

Figure S3. Overall system boundary for biomass production from microalgae cultivation with EIM and traditional culture systems.

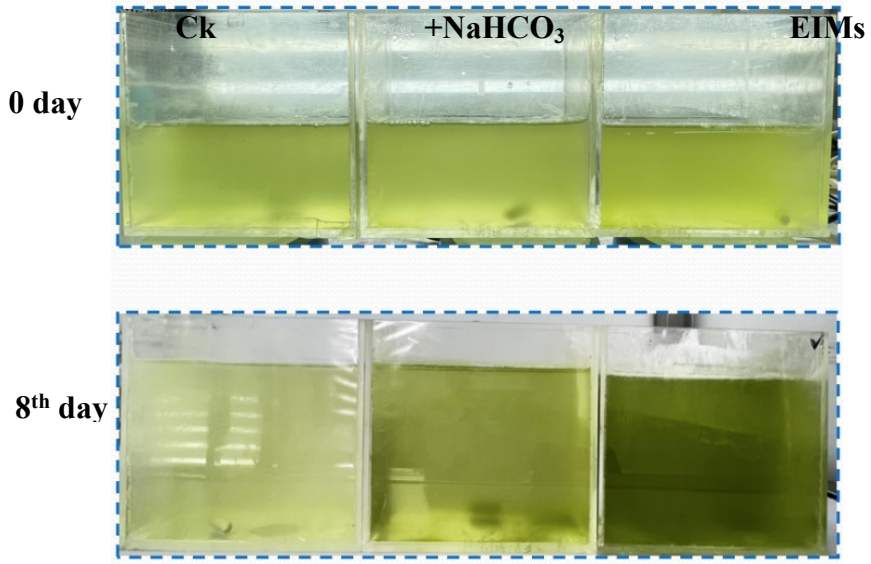
Life cycle assement (LCA) is a quantitative method for the environmental impacts and emission reduction potential from the input to the end life of the product. It was previously reported that CO_2 fixation by microalgae benefits the alleviation of global marming [1]. Here we used LCA to compare the microalgae cultivation by traditional methods or EIMs system with the developed Simapro software (https://simapro.com/).

Figure S4. Evaluation of the CO_2 Fixation in the waste catholyte



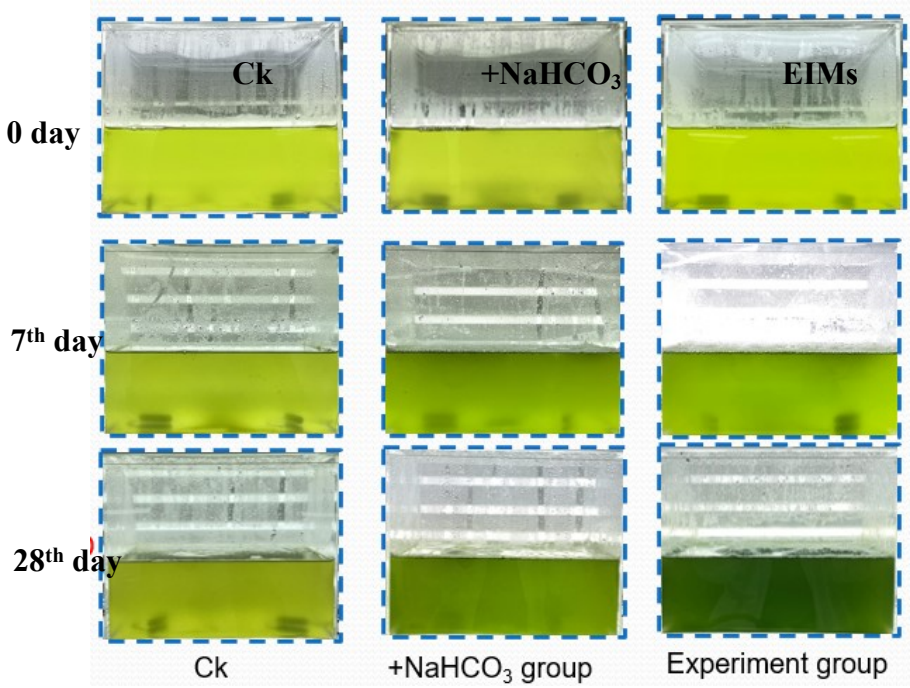
Sixty milliliters of waste catholyte was transferred into a 100 mLclosed measuring cylinder with a drain hole at the end of the cultivation, and CO_2 with 20 mL/min was aerated at the bottom of the cylinder (Fig S2A). The changes in volume were measured and are shown in Fig S2B. The CO_2 fixation efficiency (CO_2 FE) was calculated as (200-V)/200 x 100%. All results were repeated three times, and the results are expressed as the means ± standard errors of the means based on parallel experiments.

Figure S5. Visual comparison of three groups with *Chlamydomonas* strain CC-125 at days 0 and 8.



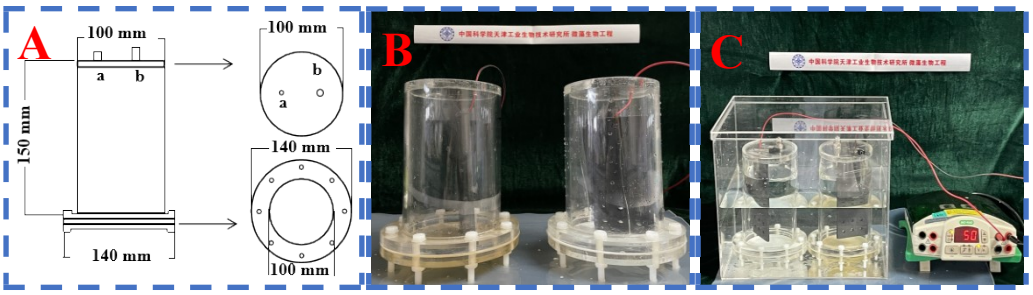
Chlamydomonas CC-125 cells were cultured to logarithmic growth phase and transferred to the microalgae pond with fresh BG11 medium. Photographs were taken on the initial day and on 8th day. The experiment was repeated at least three times, and similar results were obtained.

Figure S6. Visual comparison of three groups of *Chlorella* FA-9 at days 0, 7 and 28.



Chlorella FA-9 cells were cultured to logarithmic growth phase and transferred to the microalgae pond with fresh BG11 medium. Photographs were taken on the initial day and on the 7th and 28th days. The experiment was repeated at least three times, and similar results were obtained.

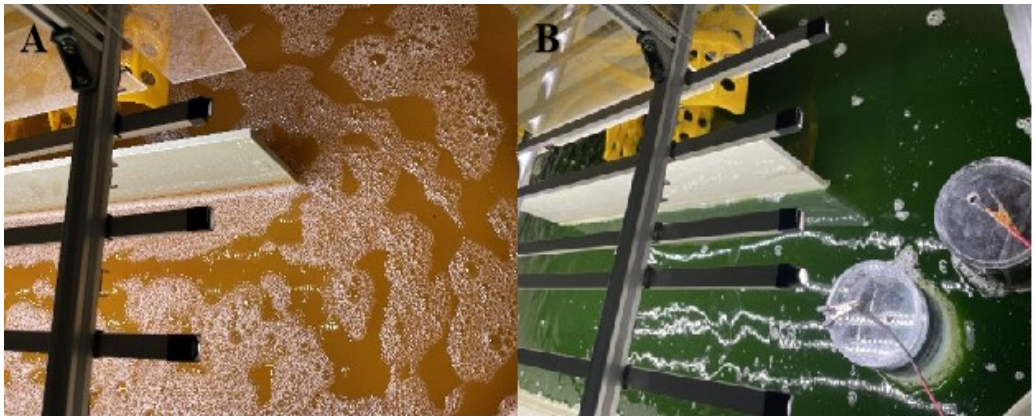
Figure S7. Assembly amplification diagram of the anode and cathode chamber in EIMs.



The electrolyte in the cathode and anode chambers easily spilled out, which might inhibit microalgae growth, as the two chambers are open. On the other hand, the small volume of electrolyte (60 mL) and area of ion exchange (35 cm^2) limited the scale-up application. Thus, a cylindrical chamber (cathode and anode) with a volume of 1000 mL and cover sealing are designed as shown in Fig. S5, where "a" and "b" represent the holes for electrical line and electrolytic gas production, respectively. As a result, carbon resources in the 1000 mL system increased 15.7-fold compared with those in the 60 mL system. To verify the potential application of EIMs in scaled and commercial production, 150 L open raceway pond microalgae cultivation was carried out. The seeds were cultured as described in the "Strains and Cultural Conditions" section, and then the cells were transferred to the racepond under 100 ± 10 μmol m^{-2} s^{-1} continuous artificial illumination at room temperature (24-26 °C) with an initial pH of 7.4 ± 0.2. The initial OD680 was adjusted to 0.1-0.2 with fresh BG11 medium. For EIM cultivation, the two chambers were placed in the medium, and the

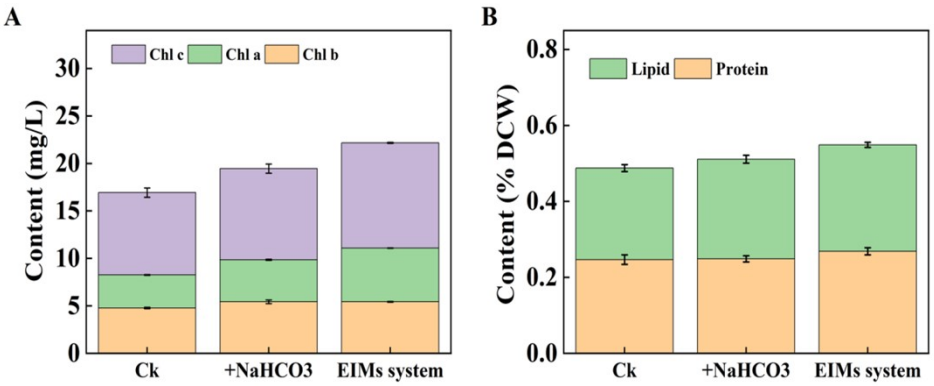
system was operated at days 4, 5, 6, 7, 9 and 13 at 120, 30, 30, 30, and 30 min and 300 mA, respectively.

Figure S8. Visual comparison of *Chlamydomonas* in the $NaHCO_3$ and EIM groups



A, Cell growth in the NaHCO3 group at day 21; B, Cell growth in the EIM group at day 21.

Figure S9. Chlorophyll (A), protein and lipid contents (B) of *Chlamydomonas* CC125



The cell precipitates were harvested at 8000 rpm for 10 min for further study. For protein analysis, the precipitates were boiled for 10 min with 0.5 M NaOH solution, the supernatant was measured using a Bradford assay. For chlorophyll detection, ice-cold 80% acetone/ethanol (v/v) was added and incubated at 4 °C overnight. For lipid content analysis, the precipitates were freeze-dried, and then approximately 40 mg of the biomass was mixed and vortexed with 6 mL chloroform-methanol (2:1, v/v). Subsequently, 2 mL methanol was added. The supernatant was obtained at 12000 rpm for 10 min and then mixed with 3.6 ml of 5% NaCl. The organic phase was then collected and transferred to a preweighed tube at 5000 rpm for 10 min and dried at 60 °C using nitrogen protection. The lipid content was calculated using the difference between the final and initial weights of the tubes. All results

were repeated three times, and the results are expressed as the means ± standard errors of the means based on parallel experiments.

Table

Table S1 Summary of environmental input and output for EIM and traditional culture systems.

Parameter	Unit	EIMs	Tradtiaonal Way
Input			
Electric energy	kWh	0.000422225	-
H_2O	g	0.096	-
CO_2	kg	1.83	18.3
$NaNO_3$	g	400.1	400.1
Na_2HPO_4	g	60.3	60.3
Output			
Microalgae biomass	kg	1	1
hydrogen	g	0.2051	-
oxygen	g	0.0256	-

Based on the typical molecular formula of microalgal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, the inputs of CO_2, $NaNO_3$ and Na_2HPO_4 are 1.83 kg, 400.1 g 60.3 g when one kg of microalgal biomass is generated, respectively. In EIMs, the supplied carbon resource was continuously replenished by CO_2 from air with the increased pH in catholyte. Total inorganic carbon supplied for microalgae fixation is 47.8 g/L per hour when an 800 mL system was used under the continuous current conditions of 10 mA. Therefore, it takes 13.04 hours to transport equal amount of carbon for microalgal capture in EIMs system. The potential difference between anode and cathode is about 3.237 V in EIM system with continuous current conditions of 10 mA. The input electric energy is calculated as W=UIT=0.000422225 kWh, whereas U represents potential difference (V), I represents electric current (A), T represents time (hour). Based on the standard Gibbs free energy of H_2O (-237.2 kJ/mol), 1 KWh of electricity could be used for the electrolyzation of 0.2267 kg of water, resulting in the production of 0.2051 kg of oxygen and 0.0216 kg of hydrogen according to the law of conservation of energy. Thus, 0.2051 g of hydrogen and 0.0256 g of oxygen is prduced in EIMs system with 0.096 g of water. In traditional production system, CO_2 is directly injected into the culture medium and more than 90% of the injected CO_2 is escaped into the atmosphere again. Therefore 18.3kg CO_2 was used when the same microalgal biomass is produced.

Table S2 Life cycle assessment per impact categories for 1 kg microalgal biomass producted by EIM and traditional culture systems.

impact category	Uint	EIMs	Traditional Way
carcinogens	Kg C2H3Cl eq	0.0263	0.0295
Non-carcinogens	Kg C2H3Cl eq	0.033	0.0369
Respiratory inorganics	Kg PM2.5 eq	0.00139	0.00173
Ionizing radiation	B1 C-14 eq	-8.2	10.1
Ozone layer depletion	Kg CFC-11 eq	5.77E-8	1.16E-7
Respiraory organics	Kg C2H4 eq	0.000158	0.000266
Aquatic ecotoxicity	Kg TEG water	134	158
terrestrial ecotoxicity	Kg TEG soil	30.4	36.2
terrestrial acid/nutri	Kg SO2 eq	0.0344	0.0406
land occupation	m2org.arable	0.0264	0.0322
Aquatic acidification	Kg SO2 eq	0.00703	0.00891
Aquatic eutrophication	Kg PO4 p-lim	0.000399	0.00891
Global warming	Kg CO2 eq	1.29	18.1
Non-renewable energy	MJ primary	6.59	18.1
Mineral extraction	MJ surplus	0.17	0.175

Table S3 Fatty acid compositions of the total lipid extract from *C. reinhardtii* cultures in different groups at 150 L

FAs	Relative content of total FAs (%, w/w)		
	Control	+$NaHCO_3$	EIMs system
C14:0	1.5±0.39	1.7±0.22	1.6±0.13
C16:0	29.4±0.19	27.8±0.42	28.8±0.51
C16:1	1.2±0.23	1.2±0.10	1.3±0.29
C16:2	2.7±0.16	2.6±0.35	2.7±0.11
C16:3	1.9±0.49	1.9±0.61	1.8±0.55
C18:0	10.4±0.39	10.2±0.12	10.7±0.11

C18:1	15.9±0.59	16.7±0.33	16.6±0.52
C18:2	10.3±0.39	11.2±0.17	10.8±0.13
C18:3	21.7±0.26	25.3±0.45	22.3±0.51
Total SFA	41.3	39.7	41.1
Total MUFA	17.1	17.9	17.9
Total PUFA	36.6	41.0	37.6
Others	5.0	1.4	3.4
Total	100	100	100

SFA represents saturated fatty acids; MUFA represents monounsaturated fatty acids; PUFA represents polyunsaturated fatty acids.

Reference

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