https://dx.doi.org/10.1016/j.cej.2022.139163

Reconfigurable modular microbiota systems for efficient and sustainable water treatment

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Abstract

Microbial synergistic interactions are key factors for carbon capture and utilization in water treatment. However, the stable and efficient synergistic interactions remains a challenge. Here, we propose a low-cost and mass-producible strategy to realize it using modular microbiota systems (MMSs). The efficient microbial synergistic interactions are based on the physical module microbiota assembly. By microfluid assisted designing, the ideal core-shell MMSs were obtained to enable good microbiota stability and efficient cycle of substance exchange. Moreover, the functionalized biomaterials immobilized MMSs possess good adsorption to the pollutant and settleability for bioresources harvesting. The practical treatment process has validated the superior performance of this strategy that the MMSs achieve an unprecedented 7-day crude treatment efficiency (10 g / L) of 95.8 %, the carbon utilization (lipid accumulation) of MMSs is elevated by 92.7 % as compared to normal algae culture treatment. And the MMSs show reliable and efficient CCU under different environment test. This approach should enable the ability to more efficient and sustainable water treatment in global settings.

Key words: Bioengineering; Carbon capture and utilization CCU; Water treatment; Engineering microbiology

1. Introduction

The paradigm shift in pollutants and wastewater treatment is an urgent need to further advance the goals of the Paris Agreement on Climate Change, due to its contribution to over 3 % of global annual greenhouse gas emissions[1-6]. For example, according to the "Urban Construction Statistical Yearbook" by Ministry of Housing and Urban-Rural Development of the People's Republic of China, the pollutants and wastewater treatment volume had reached 87.3 billion tons in 2019, and it's still increasing. The direct emissions and indirect caused emissions of greenhouse gases have become a non-negligible presence (>3%)[3,5,6]. Microorganisms as powerful natural cleaners could convert pollutants into valuable biological resources, which is expected to promote the carbon neutral and sustainability in water treatment[7-13]. And it meets the United Nations' clean development mechanism (CDM) strategic plan.

Natural microbial interactions shape the structure and function of the microbial community. In particular, positive synergistic interactions can substantially affect the treatment efficiency and productivity of natural and engineered communities. However, the efficiency and reliability of practical treatment process are affected by microbiota stability and the underutilized synergistic effects in the practical treatment. Furthermore, the high cost/energy for bioresources harvesting limits the economic benefit from treatment due to the difficult separation process of microbial biomass[3,6,14-16]. And it is an urgent need for further engineering microbiology development to realize reliable and efficient microbial synergistic interactions with low cost bioresources harvesting. Currently, a series of good studies [2,7,13,17-25] about micro-systems for particle assembly and bioenergy production are gradually emerging[26-32], which implies a potential solution to realize it via microfluidic assisted engineering microbiology[33-35].

In this work, the strategy of reconfigurable modular microbiota systems (MMSs) is proposed, it's based on physical module microbiota assembly to enhance microbial synergistic interactions for efficient CCU in water treatment (Figure 1). By microfluid assisted designing, the ideal core-shell MMSs (Core: Chlorella; Shell: Alcanivorax) were obtained to enable good microbiota stability and

efficient cycle of substance exchange. Furthermore, the functionalized biomaterials (surface modification of lipophilic groups) immobilized MMSs possess good adsorption to the oil pollutant and settleability for bioresources harvesting. We then prospectively tested the method in practical oil pollutants treatment. Our results support good performance in treatment efficiency and bioresources harvest. The proposed method should allow for the efficient and sustainable water treatment.

2. Methods

2.1. Chlorella sp. culture

The Chlorella was obtained from Freshwater Algae Culture Collection at Institute of Hydrobiology (Wuhan, China). Chlorella were cultured in BG11 medium (HAIBO, Qingdao) containing 1.76×10^{-2} M $NaNO_3$, 2×10^{-4} M K_2HPO_4, 3×10^{-4} M $MgSO_4 \cdot 7H_2O$, 1×10^{-4} M $CaCl_2 \cdot 7H_2O$, 2×10^{-4} M Na_2CO_3, 3×10^{-5} M citric acid, 2×10^{-5} M ammonium ferric citrate, 3×10^{-6} M EDTA, 4.61×10^{-2} M H_3BO_3, 9×10^{-5} M $MnCl_2 \cdot 4H_2O$, 8×10^{-4} M $ZnSO_4 \cdot 7H_2O$, 1.9×10^{-3} M Na_2MoO_4, 2×10^{-4} M $CuSO_4 \cdot 5H_2O$, 2×10^{-4} M $Co(NO_3)_2 \cdot 6H_2O$, 1 mL / L A5 solution and 919 mL / L distilled water. The medium pH was adjusted to 7.0. The Chlorella was cultured in BG11 medium at 25 °C with alternative day (12h, 3000lux) and night (12h) exposures in a light incubator (ZQLY-180V, Zhichu).

2.2. Alcanivorax culture

The Alcanivorax (97CO-5) was obtained from the Marine Ecology Research Center, First Institute of Oceanography, State Oceanic Administration. The colony of Alcanivorax was extracted from a 2216E plate medium (RISHUI, Qingdao) and transferred into liquid medium (RISHUI, Qingdao) containing 4×10^{-4} M ferric citrate, 3.325×10^{-1} M NaCl, 6.29×10^{-2} M $MgCl_2$, 2.61×10^{-2} M Na_2SO_4, 1.62×10^{-2} M $CaCl_2$, 7.4×10^{-3} M KCl, 1.5×10^{-3} M Na_2CO_3 , 7×10^{-4} M KBr, 2×10^{-4} M $SrCl_2$, 4×10^{-4} M H_3BO_3, 3×10^{-5} M Na_2O_3Si, 6×10^{-5} M NaF, 2×10^{-5} M $NaNO_3$, 6×10^{-5} M Na_2HPO_4, 5 g / L peptone and 1 g

/ L yeast powder, and incubated at 25 °C, 150 rpm / min for 48h.

2.3. Preparation of gels and lignin modified gels

1.5 % (w/w) sodium alginate (sigma) was dissolved in deionized water by stirring (35 °C, 500 rpm) for 30 min in a magnetic stirrer (MYP19-2, MEIYINPU). The 0.06 % (w/w) dealkalized lignin (aladdin, China) was dissolved in 1.5 % (w/w) sodium alginate solution by stirring at constant temperature (35 °C, 500 rpm) for 2 h in a magnetic stirrer. The gels and lignin modified gels were prepared with 2 % (w/w) $CaCl_2$ solution.

2.4. Construction of MMSs

In this experiment, the MMSs were generated based on the microfluidic generator (Figure S1). The microfluidic generator was fabricated by using the standard soft lithography process. The polydimethylsiloxane (aladdin, China) mixture composed of the silicon elastomer curing agent and silicon elastomer was poured in the silicon mold. Then, it was baked in an oven (Jinghong, XMTD-8222) at 75 °C for 1h. Then the PDMS replica was peeled off and sealed against a flat PDMS slab to form the micro-channel after oxygen plasma bonding. The microfluidic generator contains eight parallel MMSs production channels with a total throughput of approximately 30 mL / day per chip (approximately 35 million mono-dispersed MMSs). The sodium alginate solution (1.5 % w/w) with Chlorella (OD 540 = 0.9) was injected in the inlet under the flow rate of 12.8 μL / min (containing 8 parallel channels). The Alcanivorax solution was mixed in lignin modified sodium alginate solution, and injected in the inlets (Alcanivorax, OD 600 = 0.75) under the flow rate of 0.5 μL / min. Through the droplet junction, then the MMSs were produced by cross-link process with 2 % (w/w) $CaCl_2$ solution.

2.5. The practical oil treatment in seawater environment

Crude oil and seawater (table S1) were provided by the First Institute of Oceanography, State Oceanic

Administration. 10 g / L Kuwait light crude oil was added to the seawater environment. Four experimental groups were performed, including the Chlorella group (total solution: OD 540 = 0.25), Alcanivorax group (total solution: OD 600 = 0.1), Alcanivorax / Chlorella group (total solution: OD 600 = 0.1, OD 540 = 0.25) and MMSs group (approximately 150000 MMSs per ml) in the light incubator at 150 rpm / min under a light–dark regime of 14:10 (3000 lux and 25 °C). The treatment process lasts for 7 days, and the optical density, lipid content, chlorophyll-a content, GCMS analysis (alkanes and PAHs) and the crude oil degradation rate of experimental groups were measured and recorded.

2.6. Measurement of Chlorella and Alcanivorax density

The density of Chlorella was measured by OD 540 using a UV-visible spectroscopy (Analytikjena, SPECORD 210 PLUS), and the density of Alcanivorax was measured by OD 600. In the MMSs group, the MMSs were solubilized with 2 % (w/v) sodium carbonate solution (sigma), then the density of Chlorella and Alcanivorax were measured and recorded.

2.7. Measurement of the chlorella lipid content

In the experimental groups, the Chlorella collection was performed by centrifuging at 5000 rpm for 10 min and washed twice with distilled water. The Chlorella was resuspended in chloroform: methanol (2:1, v/v; sigma) and stored at 4 °C overnight, and then ultrasonic cleaning (SHUMEI, Kunshan) for 10 minutes. The freshly extracted lipids were centrifuged to obtain a clear supernatant, and the solvent was removed by evaporation under flowing nitrogen gas. After drying under nitrogen atmosphere, the lipids were gravimetrically quantified and recorded. In the MMSs group, the MMSs were first solubilized with 2 % (w/v) sodium carbonate solution (sigma), the chlorella lipid content of Chlorella was then measured and recorded.

2.8. Measurement of the Chlorophyll-a content

The Chlorella was centrifuged (5000 rpm) and the supernatant was removed, resuspending in 90 % acetone solution (sigma). And it was subsequently centrifuged again (12000 rpm, 10 min), further grinding with an ultrasonicator (HECHUANG, Kunshan) for twenty minutes, then stored at 4 °C for 12h. Chlorophyll-a was extracted from centrifuged sample and the concentration of chlorophylls-a was measured by analyzing the absorbance at 645 nm and 663 nm with a spectrophotometer. The 90 % acetone solution was used as the blank. Chlorophyll-a (Ca) concentration (mg/L) was calculated by the following equation:

$$Ca = 12.7 \times OD_{663} - 2.69 \times OD_{645}$$

In the MMSs group, the MMSs were solubilized with 2 % (w/v) sodium carbonate solution (sigma), the Chlorophyll-a content was then measured and recorded.

2.9. The GCMS analysis of alkanes and PAHs

The remaining crude oil in the four groups were freeze-dried and extracted using n-hexane (sigma) in a Soxhlet extractor (TUOFENG, Hebei). The supernatant was extracted onto C18 cartridges packed with anhydrous sodium sulfate. The saturated fraction was eluted with hexane (sigma) while the aromatic fraction was eluted with hexane in benzene (1:1, sigma). The total degradation rate, aliphatic hydrocarbon degradation rate, and polycyclic aromatic hydrocarbon (PAHs) were analyzed using GC-MS (450GC-320MS, Varian, America). Hydrocarbons were quantified by a five-point calibration curve[9,15,26].

2.10. Sample processing, DNA sequencing, and analysis

The sample were set into separate extraction bottles, and enriched for microorganisms using the 0.22 μm filter membranes (Amicrom, Hangzhou). The membranes were placed in freezing tubes then and frozen to -80 °C for DNA extraction, the high-throughput sequencing was measured using the Illumina

Sequencing Platform and the analysis were performed on the Majorbio Cloud Platform.

2.11. Characterization

Scanning electron microscopy (SEM) images were obtained on an instrument (VEGA3, TESCAN) with the samples sputter-coated with 10 nm platinum. Confocal scanning laser microscopy images were obtained on a Nikon A1R laser confocal scanning microscope. Surface profile and sphere profile of MMSs were performed on a 3D optical profiler (New View 9000, ZYGO, America). The homogeneity of MMSs was verified with a dark-field microscopy (BX53M, OLYMPUS).

3. Results

3.1. The design and characterization of MMSs

The low-cost and reproducible microfluidic technologies have been applied to generate the modular microbiota systems (MMSs). Currently, a series of high throughput generators have been reported (Telos system, UK. et al.)[17,19,24,39], which can satisfy the requirement for industrial applications. In this study, we designed the ideal core-shell MMSs with the microfluidic technology (Figure 2a), the Chlorella and Alcanivorax form a three-layer laminar flow, where the upper and lower layers are Alcanivorax, the middle layer is Chlorella, and then form the core-shell structure, recycled with 2 % (w/w) $CaCl_2$ solution (Figure 2b). The produced MMSs are composed of a core layer of Chlorella encapsulated in gels and a shell layer of Alcanivorax encapsulated in lignin modified gel, and the overall size is approximately 120 μm. The efficiency of substance exchange of microorganisms in MMSs systems determines the synergistic effect, so matching the amount of MMSs flora is necessary. The different diameter ratio (Figure 2c) of the core-shell gel structure were then tested, the results show that the 0.85 diameter ratio (core diameter / MMSs diameter) has best treatment efficiency (Figure S7), and adopted to further treatment.

The details of core-shell microbiota assembly are shown in Figure 2d. II, in this structure, Alcanivorax, as a shell layer, come into direct contact with the oil pollutants, converting them into short-chain hydrocarbons and carbon dioxide, etc. Chlorella in the core layer uses the metabolites of the bacteria for photosynthesis and produces oxygen, biological enzymes, etc. to feedback and accelerate the degradation rate of the Alcanivorax. This creates an efficient cycle of substance exchange and enhance synergistic interaction. The detailed functionalized biomaterials encapsulation (lignin modified gels) is shown in Figure 2d. III, the lipophilic groups are formed on the surface through laminar flow design[37-38] to enhance the physical adsorption of oil droplets in water. Through the scanning electron micrographs (Figure 2e) and surface profile scan (white light interference 3D surface profiler, ZYGO, America), we found that lignin modified gels has a rough surface, it provides a higher specific surface area for contaminant treatment. The MMSs image (right figure in Figure 2d. III) in oil-water mixture has validated the effectiveness of the strategy. The dark-field micrographs, light intensity analysis and color tone analysis of the MMSs were then performed. The results are shown in Figure S4, which indicate that the MMSs have good uniformity and monodispersity.

3.2. Biomass accumulation of MMSs during oil treatment

The practical treatment process was performed, including the Chlorella group (total solution: OD 540 = 0.25), Alcanivorax group (total solution: OD 600 = 0.1), Alcanivorax / Chlorella group (total solution: OD 540 = 0.25, OD 600 = 0.1) and MMSs group (the same initial biomass as in the Alcanivorax / Chlorella group, approximately 150000 MMSs per / ml) in oil contaminated (10 g / L) sea water (Aoshan Bay, Qingdao, Shandong Province). The recorded images of treatment process are shown in Figure 3a, it can be clearly seen that MMSs system have the best treatment efficiency. The fluorescent imaging analysis and biomass accumulation measurement were performed. The florescent imaging analysis of MMSs in practical treatment is shown in Figure 3b and c, there is a significant increase in the florescent intensity and area, it demonstrate the Chlorella undergoes good growth and reproduction in the MMSs

system during oil treatment. We then measured and recorded the density of Alcanivorax (OD 600) and Chlorella (OD 540), Chlorophyll A content and lipids accumulation to quantify the biomass accumulation in MMSs systems (Figure 3d-g). The results show that the 7-day average OD 600 value of the Alcanivorax group were 0.5825 with a standard deviation of 0.03983, 0.7406 value of the Alcanivorax / Chlorella group with a standard deviation of 0.03993 and 0.8608 value of the MMSs group with a standard deviation of 0.02586, these statistics indicate that MMSs possess a higher growth of Alcanivorax compared to other groups; The 7-day average OD 540 value of Chlorella group, Alcanivorax / Chlorella group and MMSs group were 0.4693 with a standard deviation of 0.03826, 0.7595 with a standard deviation of 0.04681 and 0.8837 with a standard deviation of 0.03053 respectively, these statistics demonstrate that MMSs system obtain higher Chlorella growth compared to other groups; The 7-day average Chlorophyll A values of Chlorella group were 2.704 μg / mL with a standard deviation of 0.2536 μg / mL, 4.347 μg / mL of the Alcanivorax / Chlorella group with a standard deviation of 0.2909 μg / mL and 5.268 μg / mL of the MMSs group with a standard deviation of 0.4184 μg / mL, it's clear to see that MMSs system have higher production of Chlorophyll A, which has important implications for photosynthesis; The 7-day average lipid accumulation of Chlorella group, Alcanivorax / Chlorella group and MMSs group were 0.1073 g / L with a standard deviation of 0.00818 g / L, 0.1767 g / L with a standard deviation of 0.00996 g / L and 0.2081g / L with a standard deviation of 0.01058 g / L respectively, the results show there is a significant improvement of lipid accumulation in MMSs system. Overall, the MMSs realizes more efficient biomass accumulation in treatment process.

3.3. Ultra-efficient contaminant treatment of MMSs

The gas chromatography and mass spectrometry were performed to quantify treatment efficiency. The total degradation rate, alkanes degradation rate and polycyclic aromatic hydrocarbons (PAHs) degradation rate were then analyzed, and hydrocarbons were quantified by a five-point calibration curve[9, 15, 26]. The results (Figure 4a) show that the 7-day average Alkanes residual rate of the Alcanivorax

group was 55.21 % with a standard deviation of 6.160 %, 34.56 % of the Chlorella group with a standard deviation of 5.596 %, 28.35 % of the Alcanivorax / Chlorella group with a standard deviation of 4.983 %, 3.18 % of the MMSs with a standard deviation of 2.355 %; The 7-day average PAHs residual rate (Figure 4b) of Chlorella group, Alcanivorax group, Alcanivorax / Chlorella group and MMSs group were 72.37 % with a standard deviation of 5.512 %, 46.50 % with a standard deviation of 5.271 %, 41.89 % with a standard deviation of 5.029 % and 4.96 % with a standard deviation of 3.308 % respectively; And the 7-day average total residual rate (Figure 4c) of Chlorella group was 61.07 % with a standard deviation of 5.838 %, 48.32 % of the Alcanivorax group with a standard deviation of 4.668 %, 36.51 % of the Alcanivorax / Chlorella group with a standard deviation of 3.909 % and 4.221 % of the MMSs group with a standard deviation of 2.829 %. These statistics demonstrate that the MMSs enable more efficient treatment. We then analyzed the microbiota stability of the MMSs system, and the diversity analysis was performed (Figure 4d), the results showed that the percentage of Alcanivorax in the MMSs reached 88.15 %, which demonstrates that the microbiota maintains good stability in the MMSs.

Subsequently, we performed the stability test of MMSs systems under different environments including sea water of Qingdao, Shenzhen, Sanya and Lianyungang. The key treatment efficiency and lipid accumulation were measured and recorded (Figure 5a, Figure S8a). The Bland–Altman analysis (n = 40) was then performed for comparing treatment efficience between Qingdao seawater and other three groups. As shown in Figure 5b to d, the mean bias for the treatment efficiency were -0.6359 % with a standard deviation of 3.527 % [Qingdao-Shenzhen, (LOA, from −7.518–6.276)], -0.8540 % with a standard deviation of 3.134 % [Qingdao-Sanya, (LOA, from −6.996–5.288)] and 0.5834 % with a standard deviation of 1.800 % [Qingdao-Lianyungang, (LOA, from −2.944–4.111)], these statistics show that MMSs realize efficient treatment efficiency in all four settings and there are insignificant difference in treatment efficiency under different environments, it indicates that MMSs enable stable and efficient treatment; The mean bias for the lipid accumulation (Figure S8 b to d) were -0.0003 g/L

with a standard deviation of 0.018 g/L [Qingdao-Shenzhen, (LOA, from −0.035–0.0349)], -0.0022 g/L with a standard deviation of 0.014 g/L [Qingdao-Sanya, (LOA, from −0.029–0.0246)] and 0.0046 g/L with a standard deviation of 0.011 g/L [Qingdao-Lianyungang, (LOA, from −0.018–0.027)], these statistics demonstrates the MMSs enables good lipid accumulation stability under different environments.

4. Discussion

In summary, the low-cost, scalable and mass-producible reconfigurable modular microbiota systems (MMSs) were developed for reliable and efficient carbon capture and utilization (CCU) in water treatment, which is based on enhanced microbial synergistic interactions. Through microfluidic-assisted design, the core-shell modular microbial assembly was produced to realize good microbiota stability and efficient cycle of substance exchange. The functionalized biomaterials immobilization possesses good adsorption to the pollutant and settleability for bioresources harvesting. The superior performance of this system has been verified by practical treatment process that the MMSs achieve a breakthrough 7-day crude degradation efficiency (10 g / L) of 95.8 %, while the 7-day carbon utilization (lipid accumulation) of the MMSs has greatly improved from 0.1079 g / L to 0.2081 g / L compared to the normal Chlorella culture treatment. This strategy should improve CCU in water treatment industry.

Along with the continuous industrialization and urbanization process, the pollutant and wastewater treatment industry contributes more than 3% of greenhouse gas emissions and hundreds billions dollars cost, and it is rapidly growing[41-44]. Microorganisms as powerful natural cleaners could convert pollutants and waste into valuable biological resources (clean water, biofuel and feed, etc.), have potential to transform energy-intensive, carbon-emitting water treatment into integrated water resource recovery with economic, environmental and social benefits[34, 45-46]. The current challenges mainly focus on that it needs to meet and balance multiple objectives to fulfil different treatment needs, and realize reliable and

efficient microbial synergistic interaction to improve CCU in water treatment[3, 6]. This work innovatively propose a low-cost and mass-producible strategy via microfluidic assisted modular microbiota assembly to realize it. Moreover, this system possesses good reconfigurability to meet different treatment need. The further promotion and applications are expected to advance the carbon neutral in water treatment, and provide sustainability gains and carbon trading over hundreds billion market scale.

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Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 62175190), Foundation Research Fund of Shenzhen Science and Technology Program (No. JCYJ20190808154409678).

Author contributions

Longfei Chen and Yi Yang conceived and designed the research. Longfei Chen, Yantong Liu, Le Yu, Fang Wang and Yifan Wang performed the experiments. Yantong Liu, Wei Li and Hongshan Xu helped microfluidic chip production. Fenghua Jiang and Li Zheng provided the seawater and microorganisms. Li Zheng, Xuming Zhang and Chengjun Sun helped with data analysis. Longfei Chen, Yantong Liu and Pengfu Tian visualized this work. Longfei Chen and Yantong Liu wrote the original draft. Xuming zhang and Yi Yang reviewed and edited the draft. Yi Yang supervised and coordinated all of the work. All authors contributed to the interpretation of the findings.

Data availability

All data needed to evaluate the conclusions are present in the Manuscript and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Competing interests

The authors declare no competing interests.

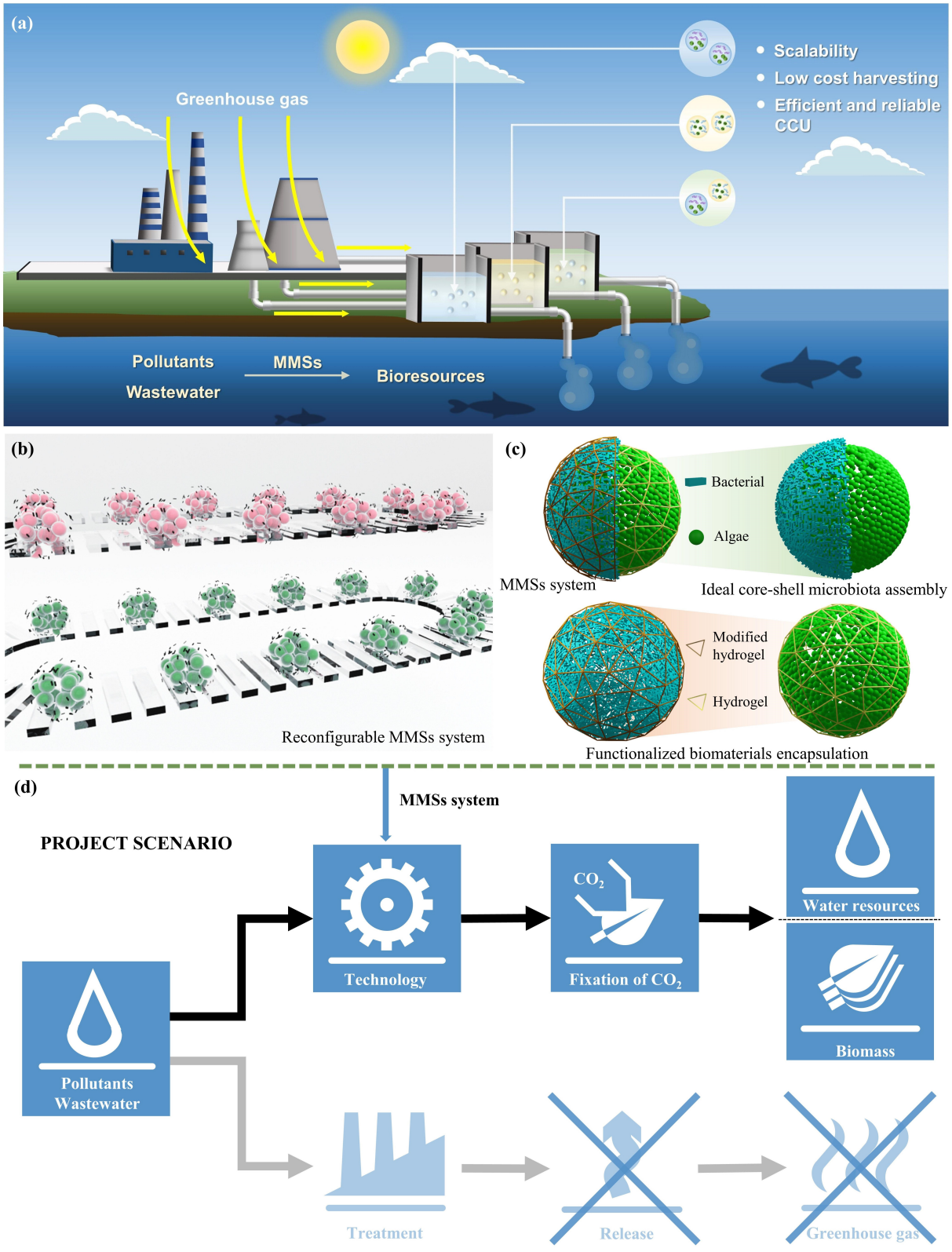

Figure 1. **(a)** The schematically applications and advantages of reconfigurable modular microbiota systems. **(b)** The schematically diagram of mass modular microbiota systems. **(c)** The schematically diagram of reconfigurable modular microbiota systems' detailed structure. **(d)** The project scenario of further MMSs applications (icons from United Nations' Clean Development Mechanism).

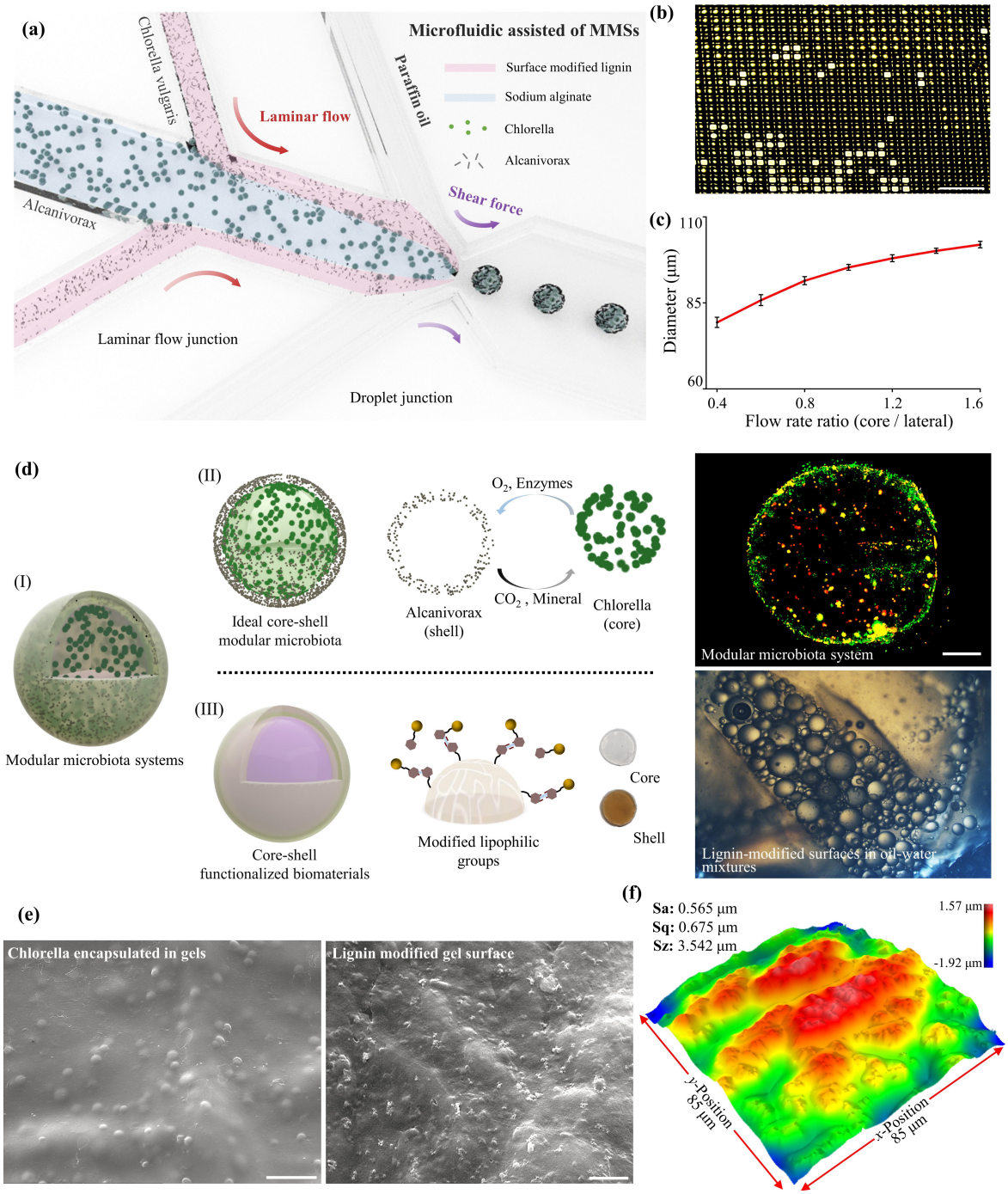

Figure 2. The design and characterization of MMSs. **(a)** The schematic diagram of microfluidic assisted reconfigurable modular microbiota systems production. **(b)** The micrograph of MMSs, scale bar is 500 μm. **(c)** The diameter ratio manipulation of core-shell MMSs via flow rate adjustment. **(d)** The dual core-shell structural (Chlorella-Alcanivorax; gels-lignin modified gels) of MMSs, scale bars are 30 (upper figure) and 8 (lower figure) μm. **(e)** Scanning electron microscopy characterization of gels and lignin modified gels, scale bars are 20 (left figure) and 5 (right figure) μm. **(f)** Surface profile analysis of lignin modified gels.

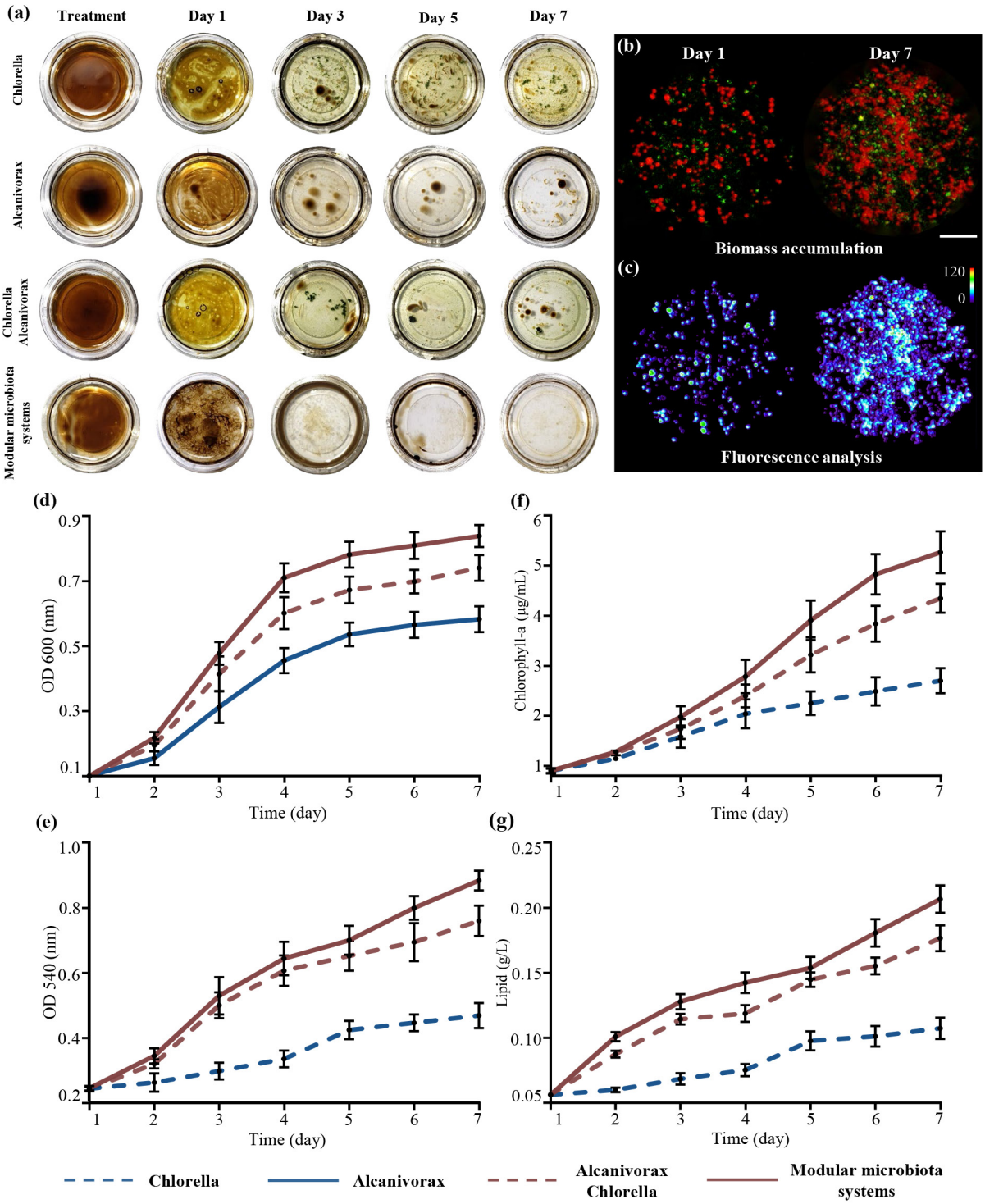

Figure 3. Biomass accumulation of MMSs in practical treatment. **(a)** The recorded images in practical treatment process. **(b-c)** The fluorescent characterization of Chlorella growth in MMSs and fluorescence intensity analysis, scale bar is 30 μm, color bar refers to the light intensity. **(d-g)** Density of Alcanivorax and Chlorella, Chlorophyll-A and lipid content in the treatment process.

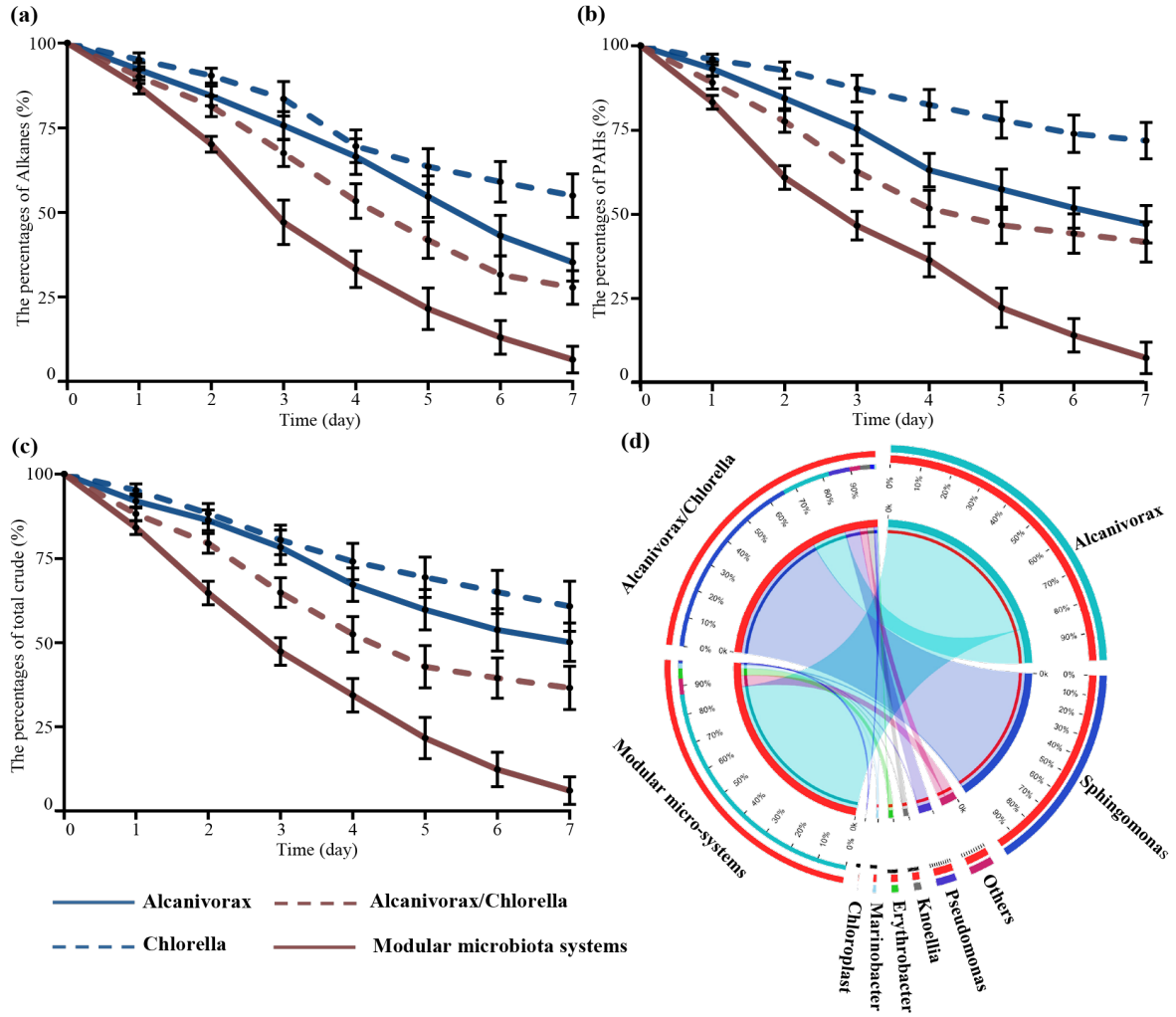

Figure 4. Ultra-efficient oil contaminant treatment of MMSs. **(a-c)** The total degradation rate, Alkanes degradation rate and polycyclic aromatic hydrocarbon (PAHs) degradation rate of experimental groups in 7 days. **(d)** The diversity analysis between Alcanivorax / Chlorella group and MMSs.

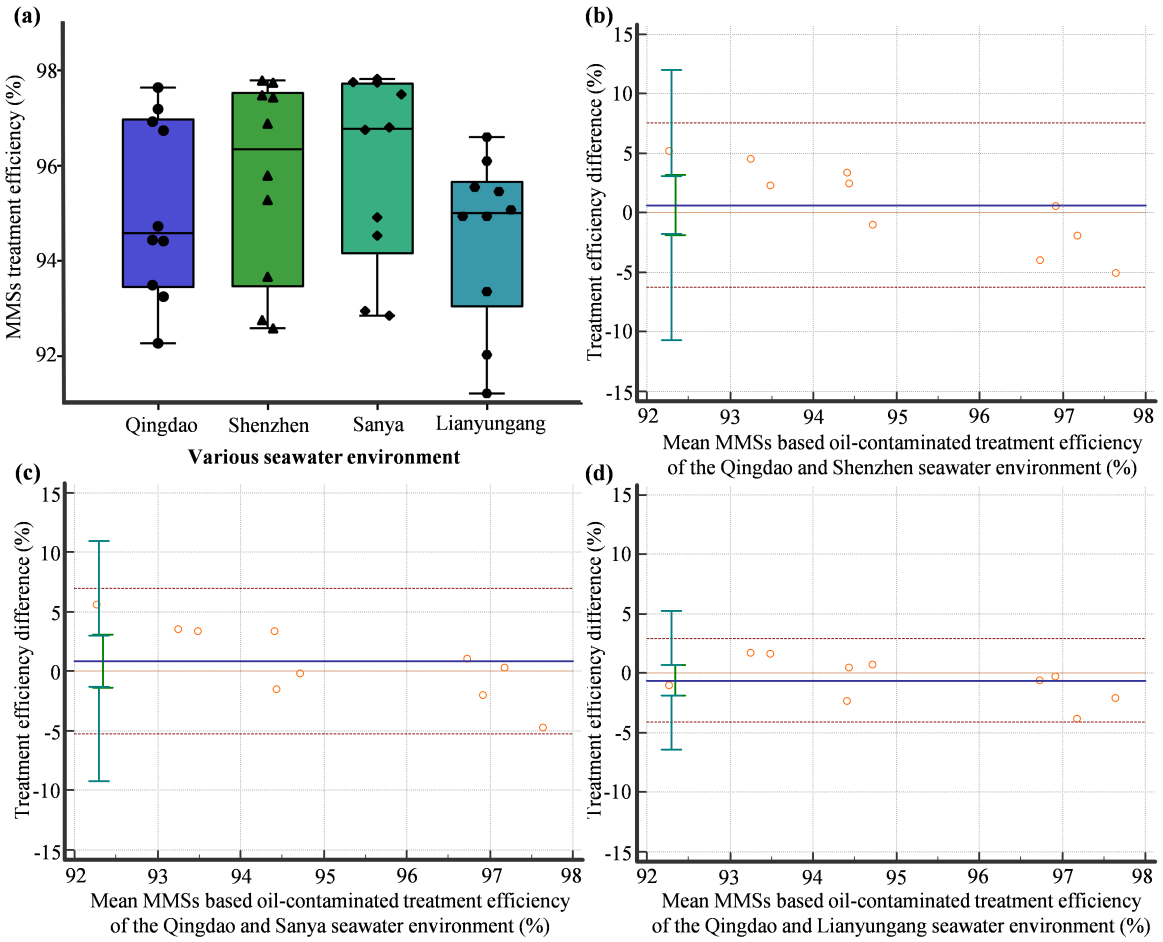

Figure 5. The oil pollutant treatment of MMSs under different seawater environments. **(a)** The total treatment efficiency of MMSs under different seawater environments. **(b-d)** The Bland–Altman analysis for evaluating the performance of the MMSs in different seawater environments.

Supplementary Table

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: