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研究課題名（和文）A Sustainable and Recycling-oriented Wastewater Industry by Maximizing Valuable Resources and Energy Recovery Using the Novel Algal-bacterial Aerobic Granular Sludge Technology

研究課題名（英文）A Sustainable and Recycling-oriented Wastewater Industry by Maximizing Valuable Resources and Energy Recovery Using the Novel Algal-bacterial Aerobic Granular Sludge Technology

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研究成果の概要（和文）：下水処理場を持続的に発展させるため、排水からの資源・エネルギー回収が非常に重要である。本研究では、以下のように実りある成果が得られた。(1)天然および単細胞微細藻類の速やかなグラニュール化と収穫、(2)リン、アルギン酸塩様エキソポリマー(ALE)、タンパク質、多糖類、脂質等付加価値物質の蓄積と生産、(3)運転モードによるグラニュールの安定性と栄養素除去への影響、(4)異なる環境条件下でグラニュールの安定性と浄化効果、(5)重金属（クロム）の回収と重金属負荷グラニュールの安全な後処理、(6)共生細菌/微細藻類および単一微細藻類種のグラニュール化と細胞外高分子物質（EPS）の機能への洞察。

研究成果の学術的意義や社会的意義
During 2018-2022, we published 37 articles in the top 10 jourals in the field of Environmental Engineering, and shared 36 presentations (5 invited keynotes) with peers in international conferences. The results definitely lay a solid foundation for the sustainable management of wastewater industry.

研究成果の概要（英文）：Resource/energy recovery from wastewater is crucial for sustainable development of wastewater treatment plants (WWTPs). In this project, we achieved fruitful results on the following six aspects: (1) Rapid granulation and harvesting of natural microalgae and single cell microalgae; (2)Value-added biomaterials accumulation and production from wastewater, including phosphorus, alginate-like exopolymers (ALE), proteins, polysaccharides, and lipids; (3) Impacts of operation mode on the system stability and nutrients removal; (4) Granular system stability and remediation under various conditions; (5) Heavy metal (with Cr as example) recovery from wastewater and safe posttreatment of heavy metal-loaded granules; and (6) Deep insight into the biogranulation of bacteria/microalgae symbiosis and microalgae, highlighting the functions of specific microalgae species and extracellular polymeric substances (EPS).

研究分野：環境工学

キーワード：Algal-bacterial granule　Bacterial grannule　Oil producing microalgae　Microalgae granulation　Phosphorus recovery　Heavy metal　Energy production

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１．研究開始当初の背景

Energy shortage, resource depletion and greenhouse gases (GHGs) like CO_2 emission are major concerns which profoundly restrain the sustainable development of wastewater treatment plants (WWTPs). Resource and energy recovery from wastewater nationwide is still at a relatively low level in Japan, probably due to the low contents of target resource elements accumulated in the produced biomass thus low profitability for their recovery.

In this context, algal-bacterial symbiotic system is regarded as one of the most promising alternatives. This system can not only achieve up to 100% removals of target pollutants without mechanical aeration, the most energy-intensive step in the conventional activated sludge process (ASP)-based WWTPs, but also consume much less energy with less CO_2 emission (Praveen and Loh, 2015; Ramanan et al., 2016; Quijano et al., 2017). Besides, the chemical energy in wastewater can be preserved in the grown biomass for further reclamation. However, this technology has not been developed as fast as expected, most probably due to the costly biomass separation/harvesting and unstable biomass quality for further resource and energy recovery.

On the other hand, bacterial aerobic granular sludge (AGS) process can effectively remove nitrogen (N) and phosphorus (P), and accumulate P and metals from wastewater with P-rich (6-15%) granules produced during wastewater treatment (Li et al., 2014; Cai et al., 2016; Huang et al., 2014; Huang et al., 2015a, 2015b). Moreover, naturally grown algae can interact with bacteria to successfully form stable algal-bacterial AGS under natural sunlight (Huang et al., 2015c). This symbiotic system can also be re-established by seeding mature bacterial AGS and stably operated in continuous-flow reactors under uncontrolled room light condition (Ahmad et al., 2017). These precious observations suggest its great potentials for efficient pollutants removal and resource recovery in the real world of wastewater treatment. In addition, with the co-existence of algae, this symbiotic system can remove or uptake more pollutants or resources including N, P, heavy metals (HMs) and other toxic organics (Ramanan et al., 2016; Quijano et al., 2017), achieving better effluent quality and more accumulation of value-added resources into the grown biomass. Up to the present, however, very limited information is available on algal-bacterial AGS, not to mention how to use this new symbiotic system to realize efficient energy and resource recovery from wastewater.

In this project, it is speculated that this new algal-bacterial symbiotic granule system, or algal-bacterial AGS is definitely the most promising biotechnology for wastewater treatment. By using this new technology, large reductions in energy consumption (due to no need for mechanical aeration) and CO_2 emission can be achieved in addition to easily harvested microalgal granule biomass for further resource and energy recovery.

２．研究の目的

This project focused on resources and energy recovery from wastewater via the novel algal-bacterial AGS process, targeting the sustainable management of WWTPs. The main purposes of this proposed research are as follows: (1) to identify the main contributors to the formation of algal-bacterial AGS and its quick formation condition; (2) to recover resources (P, biopolymers, HMs) from wastewater via algal-bacterial AGS system; (3) to explore the formation of microalgae granules from natural microalgae and functional microalgae and their dynamic response to the changes of nutrients loading and the potentials for energy and resource recovery; and (4) to estimate the benefits from the algal-bacterial AGS-based WWTP, contributing to the sustainable wastewater industry.

３．研究の方法

(1) All the experiments were performed in 8 ×1L and 2×16L algal-bacterial AGS reactors in comparison with bacterial AGS and/or activated sludge-based systems. Before these tests, the characteristics of influent wastewater to the local Shimodate WWTP were analyzed, mainly including pH, chemical oxygen demand (COD), total N and P and their speciation, HMs, and each element, etc. This basic information was used for preparation of synthetic wastewater in this project according to our previous works (Yu et al., 2017; Huang et al., 2014; Huang et al., 2015a). All the analyses were carried out according to these previous works. Bacterial and microalgal communities were analyzed by 16S and 18S rDNA sequencing after DNA extraction and amplification. The impacts of changes in influent characteristics (especially organic composition like fatty acid species, ionic composition), iron species and dosage, operation mode (batch or continuous), seeding granules or not, different light illuminance and strategies, under air bubbling or no air bubbling, etc. on the rapid formation of algal-bacterial AGS, stability, and system performance were specifically explored.

(2) Main resources including P, HMs (with Cr as an example), biopolymers (extracellular polymeric substances (EPS), alginate-like exopolymers (ALE)) were quantitatively recovered and compared from bacterial and algal-bacterial AGS systems. CO_2 emission reduction potential was also recorded in the case of algal-bacterial AGS systems.

(3) The formation of microalgae granules was performed by using the isolated natural microalgae from the matured algal-bacterial AGS and the oil producing single cell microalgae (*Ankistrodesmus falcatus var. acicularis*) to confirm the feasibility of the biogranulation process proposed in this project and their functions during the formation and stable operation of granule system. Much attention was paid to granule development, size distribution, stability, and algae content (indicated by chlorophyll a/b/c) in addition to pollutants removal, resource accumulation, and energy consumption/recovery.

(4) Estimation and comparison were made on resource and energy recovery from bacterial AGS, algal-bacterial AGS and microalgal granule systems. The information of pollutants removal, biomass production, resource accumulation, biogas (H_2, CH_4, CO_2) production from the grown biomass, and energy balance (consumption/recovery) were collected and calculated, which were used for the estimation of resource and energy recovery from the proposed and scaled-up

wastewater treatment system according to the influent characteristics in the local Shimodate WWTP.

４．研究成果

During the four years' research works, we achieved fruitful results on the following six aspects.

(1) Realized the rapid granulation and harvesting of natural microalgae and single cell microalgae *Ankistrodesmus falcatus var. acicularis*, and the latter can be used for highly efficient biomaterials production from wastewater treatment.

(2) Successfully recovered and compared the production of the value-added materials from bacterial and algal-bacterial AGS systems treating wastewater, including P, alginate-like exopolymers (ALE), proteins, polysaccharides, and lipids according to the treatment capacity of the proposed WWTP. And the energy content of algal-bacterial AGS was found to be much higher (> 80%) than that of bacterial AGS under the tested no air bubbling conditions.

(3) Made clear the impacts of different operation modes and light strategies on the granular system stability and nutrients removal performance, in addition to the response of algal-bacterial AGS to different ionic strength and commonly co-existing ions like $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, etc. in the influent, especially during phosphorus removal and recovery via algal-bacterial AGS.

(4) Understood the granular system stability under various environmental conditions and proposed efficient remediation methods to cope with the instability issue.

(5) Heavy metal (with Cr as example) was also removed and recovered from wastewater via algal-bacterial AGS system, and the heavy metal-loaded AGS was hydrothermally treated to achieve its safe posttreatment with mobility factor of 0. And

(6) gained a deep insight into the biogranulation of bacteria/microalgae symbiosis and microalgae systems, highlighting the key functions of specific microalgae species and extracellular polymeric substances (EPS).

Based on the above results, we further figured out the major differences between the algal-bacterial AGS systems respectively with the natural microalgae and oil-producing single cell microalgae (i.e., *Ankistrodesmus falcatus var. acicularis*) in terms of pollutants removal, resources and energy recovery from biomass, and their dynamic changes in microbial biodiversity. Moreover, the interactions between microalgae and bacteria including ionic response, O_2 generation, CO_2 sequestration potential, intermediate products, and microbial biodiversity were addressed. All the results will help better design and stably operate the algal-bacterial AGS systems, serving for the subsequent resources and energy recovery from the grown biomass.

During the four years' research, we totally published 46 journal articles and 36 presentations in domestic/international conferences to share our new knowledge on this novel wastewater biotechnology with the peer researchers worldwide. Among them, 37 articles were published in the top 10 journals in the field of Environmental Engineering, and 5 presentations were invited keynotes. Through editing one special issue (granular consortium of bacteria and microalgae) on the high-quality journal *Bioresource Technology* (IF=9.642, ranking the top 2 journal in the field of Environmental Engineering), we also established a global network with more than 50 institutions from 13 countries on granular consortium of bacteria and microalgae (GCBM) for energy and resource recovery.

Results from this project would definitely lay a solid foundation for the sustainable management of WWTPs where can be transformed from traditional energy/resource consumer to energy/resource producer, contributing to the establishment of the sustainable society. This project is much meaningful to Japan where has a low energy/resource self-sufficiency.

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掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.biortech.2022.127104	査読の有無 有
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関