氏　　名	王 倩 (WANG QIAN)
学位の種類	博　士（ 環境学 ）
学位記番号	博　甲　第　10116　号
学位授与年月日	令和　3　年　9　月　24　日
学位授与の要件	学位規則第４条第１項該当
審査研究科	生命環境科学研究科

学位論文題目 Biogranulation Process for Simultaneously Quick Harvesting of Oil-producing Microalgae and Digestion Liquor Treatment
(バイオグラニュールプロセスを用いた油生産微細藻類の速やかな収穫及び発酵消化液処理)

主査	筑波大学教授	博士（農学）	張　振亜
副査	筑波大学准教授	工学博士	雷　中方
副査	筑波大学准教授	博士（農学）	清水　和哉
副査	筑波大学准教授	博士（工学）	Helmut Yabar

論　文　の　要　旨
Abstract of thesis

Microalgae-based technologies have attracted lots of interests owing to their high efficiencies on simultaneous wastewater remediation and nutrients recovery, and production of alternative biofuels or other high-value products. However, microalgae biomass harvesting is still the bottleneck for its large-scale application, which accounts for 20 - 30% of the operation cost for microalgae cultivation. Although several methods such as centrifugation, filtration, coagulation and flocculation have already been applied for microalgae harvesting, till today none of them has been proven economical and efficient at large scale. Previous studies show that microalgae and bacteria can form granular symbiosis consortia through biogranulation, *i.e.*, algal-bacterial aerobic granular sludge (AGS). In this study, the feasibility of granule formation was investigated by using unicellular oil-producing microalgae *Ankistrodesmus falcatus* var. *acicularis* for simultaneously quick harvesting of biomass and digestion liquor treatment in lab-scale sequencing batch reactors (SBRs). Additionally, the mechanisms involved in microalgae biogranulation and its cost-effectiveness analysis were carried out towards practical application.

This dissertation is divided into 5 chapters. In Chapter 1, the author introduced the research background and literature review. In this chapter, the author discussed the existing problems relating to microalgae-based technologies for wastewater purification and resource recovery, especially those from cultivation and harvesting process. Specifically, the author addressed the great potentials of biogranulation as a novel alternative for non-suspended cultivation, and then arrived at the objectives and framework of this research. In Chapter 2, the author investigated the feasibility of biogranulation using unicellular oil-producing microalgae *Ankistrodesmus falcatus* var. *acicularis* for fast microalgae harvesting and nutrients removal from anaerobic digestion liquor. Results show that easily settled microalgal granules with compact structure appeared around day 90 and mature granules were obtained after 150 days' operation. The microalgae settleability was remarkably improved, signaling by the substantial decrease of sludge volume index (SVI_{30}) from initially > 3000 to 53.44 ± 3.31 mL/g, with settling velocity correspondingly increased from nearly 0 to 18.47 ± 0.23 m/h. Although the percentage of the target microalgae (*A. falcatus* var. *acicularis*) decreased along with the granulation process, the biomass concentration (2 - 4 g/L) and biomass productivity (130 - 270 mg/L/d) using biogranulation were 10 - 20 times

and 16 - 34 times than that by the traditional suspension method. Compared to the seed microalgae cells, more extracellular polymeric substances (EPS) (162.54 ± 3.60 mg/g-volatile suspended solids (VSS)) with a higher proteins/polysaccharides ratio (7.62) were excreted from the mature microalgae granules. Moreover, the mature microalgae granules showed comparable nutrients removal, averagely 96% and 86% of dissolved organic carbon (DOC) and ammonia nitrogen (NH_4^+-N) removal from the digestion liquor, reflecting its great potential for simultaneous microalgae cultivation, harvesting and wastewater treatment. In Chapter 3, the author utilized the mature microalgae granules to quickly harvest microalgae and explored the mechanisms involved. Results show that the aggregates ranging from 0.5 to 2 mm with excellent settling capability were obtained in the group with liquid extract (from crushed granules) as additive, achieving harvesting efficiency about 90.75 ± 1.23% in 30 min. The continuous increase in polysaccharides content in soluble EPS might be responsible for the cells capture and adhesion at the early stage of aggregation, while the increase in bound EPS content from 41.86 ± 5.08 mg/g-VSS to 264.05 ± 6.41 mg/g-VSS favored the stabilization of aggregate integrity, in which 93.8% was attributed by the tightly bound EPS. The aromatic proteins in loosely bound EPS as well as tightly bound EPS were dramatically enhanced in response to the liquid extract addition, and N-acyl-homo-serine lactone mediated quorum sensing might be involved in this granulation process. In Chapter 4, the author conducted the comparison between this novel biogranulation system and the conventional suspended cultivation for the cultivation and harvesting of the oil-producing microalgae (*A. falcatus* var. *acicularis*) with respect to biomass accumulation, energy consumption and nutrients removal efficiency towards practical application. Results revealed that the biogranulation system achieved enhanced volumetric biomass productivity (223.17 ± 11.82 g/m^3/day) by 4.4 times when compared to the suspended system (41.57 ± 2.08 g/m^3/day) under the same environmental conditions. The biomass mass-based biofuel production was comparable between the two systems. More importantly, use of biogranulation improved the volumetric energy production by 3.3 times (3.44 ± 0.29 MJ/m^3/day versus 0.81 ± 0.06 MJ/m^3/day in the suspended system). Moreover, the biogranulation system can reduce biomass mass-based electricity consumption by 58% and footprint demand by 76%, respectively, demonstrating its great superiority in microalgae biomass cultivation and harvesting in large scale application. Finally, in Chapter 5, the author summarized the major conclusions, and proposed the future research directions.

審　査　の　要　旨

Abstract of assessment result

This research established a novel biogranulation method for single cell microalgae cultivation and harvesting, contributing to scalable microalgae-based wastewater treatment and biofuel production system. Under the proposed operation strategy, mature microalgae granules were obtained with excellent settleability and nutrients removal performance while efficiently treating digestion liquor (up to 96% of organics and 84% of NH_4^+-N removal). Comparison analysis was also conducted between this novel biogranulation system and conventional suspended cultivation under the same environmental conditions, suggesting that the biogranulation system could achieve much higher volumetric biomass productivity and energy production with reduced biomass mass-based electricity consumption and footprint demand. Moreover, much quicker harvesting and aggregation of microalgae suspension was also realized by addition of liquid extract from crushed mature microalgae granules, shedding light on the mechanisms involved in microalgae granulation and the major contributors to this quick cultivation and harvesting technology for microalgae. The novel biogranulation system established in this study possesses a high potential for cost and land requirement reduction in microalgae cultivation and harvesting, paving the way for the application of microalgae-based wastewater treatment in practice. More attention should be paid to the changes of valuable components in the target microalgae along with the granulation process.

The final examination committee conducted a meeting as a final examination on 12 July, 2021. The applicant provided an overview of the dissertation, addressed questions and comments raised during Q & A session. All of the committee members reached a final decision that the applicant has passed the final examination.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.