

**Bi granulation Process for Simultaneously Quick
Harvesting of Oil-producing Microalgae and Digestion
Liquor Treatment**

July 2021

WANG QIAN

**Bi granulation Process for Simultaneously Quick
Harvesting of Oil-producing Microalgae and Digestion
Liquor Treatment**

A Dissertation Submitted to
the Graduate School of Life and Environmental Sciences,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Environmental Studies
(Doctoral Program in Sustainable Environmental Studies)

WANG QIAN

Abstract

Resource depletion and environmental pollution issues have been focused recently along with the rapid increase of global population. Thus, the exploration of environmentally friendly technologies that facilitate waste treatment and resource recovery is an essential part for sustainable development. Recently, microalgae-based technologies have attracted lots of interests owing to its high efficiency on wastewater remediation and simultaneous nutrients recovery, and production of alternative biofuel 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. Though several methods such as centrifugation, filtration, coagulation and flocculation, etc. have already been applied for microalgae harvesting, till today none of them has been proven economical and efficient at large scale. Further investigation and development of large-scale applicable harvesting methods is necessary for microalgae-based wastewater treatment or bioproducts production.

Previous studies revealed 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 using unicellular oil-producing microalgae *Ankistrodesmus falcatus* var. *acicularis* for simultaneously quick harvesting of biomass and digestion liquor treatment was investigated in lab-scale sequencing batch reactors (SBRs). Additionally, the mechanisms involved in microalgae biogranulation, and its cost-effectiveness analysis were carried out. The main results can be summarized as follows.

(1) Easily settled algae 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 (SVI₃₀) 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 algae granules. Moreover, the mature microalgae granules showed comparable nutrients removal, averagely 96% and 86% of dissolved organic carbon (DOC) and ammonia nitrogen

(NH₄⁺-N) from the digestion liquor, reflecting its great potential for simultaneous microalgae cultivation, harvesting and wastewater treatment.

(2) Aggregates ranging from 0.5 to 2 mm with excellent settling capability were obtained in the group with liquid extract as additive, achieving harvesting efficiency in 30 min about $90.75 \pm 1.23\%$. The continuous increase in polysaccharides in soluble EPS might be responsible for cells capture and adhesion at the early stage of aggregation, while the increase in concentration of bound EPS from 41.86 ± 5.08 mg/g-VSS to 264.05 ± 6.41 mg/g-VSS favored the stabilization of aggregates integrity, in which 93.8% was attributed by 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, and N-acyl-homo-serine lactone (AHLs) mediated quorum sensing might be involved in this granulation process.

(3) Comparison in cost effectiveness between this novel biogranulation system and the conventional suspended cultivation for oil-producing microalgae (*A. falcatus* var. *acicularis*) cultivation and harvesting was conducted in regard of 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/m³/day) by 4.4 times when compared to the suspended system (41.57 ± 2.08 g/m³/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/m³/day versus 0.81 ± 0.06 MJ/m³/day in the suspended system). Additionally, 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 for large scale application.

The findings from this work are expected to provide the guideline for a cost-effective and sustainable cultivation and harvesting approach for scalable microalgae-based wastewater treatment and biofuel production. This work also sheds light on the mechanisms involved in microalgae granulation and the major contributors to this novel cultivation and harvesting technology for microalgae.

Keywords: Microalgae harvesting; Wastewater treatment; Biofuel production; *Ankistrodesmus falcatus* var. *acicularis*; Extracellular polymeric substances