Techniques for mass-culturing microalgae using anaerobic digestion effluent from over-growing and invasive aquatic macrophytes

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General Introduction

Aquatic macrophytes play an important role as a one of the functional components in aquatic ecosystems, but recently have been excessively grown and led to various ecological and economic problems all over the world. It is difficult to control or eliminate these overgrown macrophytes with chemical or biological agents owing to high costs. Anaerobic digestion (AD) is one of the effective and low-cost bioenergy recovery technology employing the consortia of anaerobic microorganisms for the treatment of organic wastes, including aquatic macrophytes harvested.

Anaerobic digestion effluent (ADE) containing high concentrations of nutrients such as nitrogen (N) and phosphorus (P) may serve as an inexpensive nutrient-rich medium for growth of microalgae. Recently, microalgae have been widely applied for nutrients removal from ADE. Since NH_4^+ is major form of nitrogen in ADE, dilution of ADE is necessary prior to algal cultivation in order to avoid the potential inhibition for algal growth due to high NH_4^+ concentration. However, diluted ADE may result in insufficient amounts of available nutrients, such as magnesium (Mg), for algal growth. Actually, Mg ion (Mg^{2+}) is one of essential elements for algal growth and cell division. It has been shown that available Mg^{2+} was insufficient for algal growth in 10-fold diluted ADE from aquatic macrophytes, although Mg itself is not scarce. Therefore, it is needed to identify chemical speciation of Mg in ADE, which affect Mg uptake by microalgae. Nitrification is another solution for high ammonium (NH_4^+) toxicity in ADE, and can be achieved by changing the oxidation state of nitrogen from NH_4^+ to nitrate (NO_3^-) as a more amenable and non-toxic form.

Several microalgae such as green algae efficiently remove nutrients from wastewater, due to assimilating the abundant N and P that serve as nutrients for their growth. However, biomass yield level and nutrient removal efficiency differ across microalgae species in wastewater treatment. *Chlorella sorokiniana* and *Chlamydomonas reinhardtii* were widely used for mass-culturing with wastewaters including ADE, and showed high level of algal biomass yields and nutrient removal efficiency. *Botryococcus braunii* can synthesize and accumulate an high level of hydrocarbons, such as lipids.

Chapter 1

In this Chapter, the three green algae, *C. sorokiniana, C. reinhardtii and B. braunii,* were cultured in two different ADEs from invasive macrophyte *Ludwigia grandiflora* (LADE) and co-digestates of submerged macrophytes and food wastes (CADE), to determine suitable treatments of each ADE for reaching maximum algal yield in and nutrient removal from the ADEs, and selected the most suitable species for each of the ADEs.

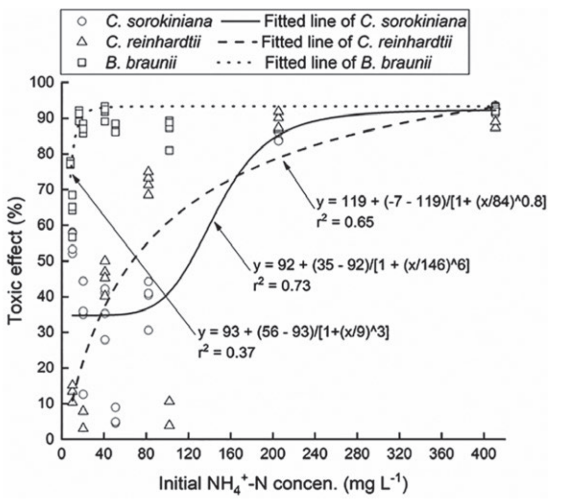

Fig. 1 Toxic effect of NH_4^+-N concentration on algal growth for cultivation of *C. sorokiniana* (circles), *C. reinhardtii* (triangles) and *B. braunii* (squares) in CADE and LADE.

NH_4^+ tolerances on half toxic effect were 146, 84 and 9 mg L^{-1} in both ADEs among the dilution series for growth of *C. sorokiniana, C. reinhardtii* and *B. braunii*, respectively. The most suitable dilutions for cultivation of *C. sorokiniana, C. reinhardtii* and *B. braunii* were therefore in 5×, 10× and 50× diluted CADEs, respectively, while in undiluted, 2× and 10× diluted LADEs. Insufficient amounts of available Mg for reaching maximum algal yield and nutrient removal existed in the 5×, 10×, 50× diluted CADE, respectively (also 10× diluted LADE for B. brauni). The minimum concentrations of Mg at 57 and 12 mg L^{-1} in ADEs were sufficient for the maximum growths of *C. sorokiniana* and *C. reinhardtii*, respectively. *C. sorokiniana* in each ADE under sufficient Mg showed the highest algal biomass yields (optical density (OD) = 1.08-1.16) and nutrient removal rates (> 90%) among the three species tested. C. sorokiniana can not only obtain high algal biomass yield, but also have great abilities of high nutrient removal and NH_4^+-N tolerance to ADE.

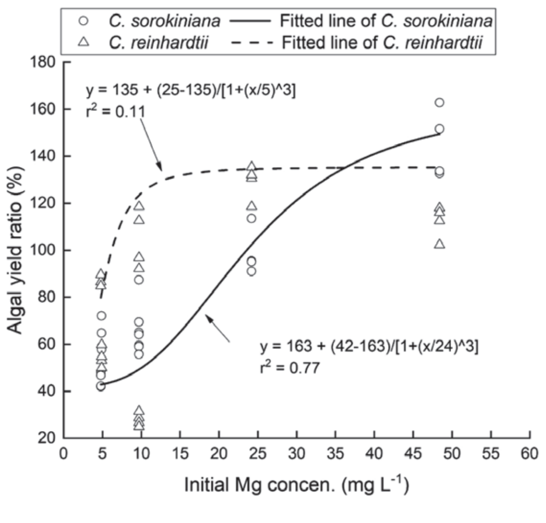

Fig. 2 Relationship between algal yield ratio and initial Mg concentrations for cultivation of *C. sorokiniana* (circles) and *C. reinhardtii* (triangles) in CADE and LADE.

Chapter 2

In this study, we determined suitable pH and hydraulic retention time (HRT) levels for continuous cultivation of *C. sorokiniana*, using ADE from aquatic macrophytes to permit maximum algal biomass production and nutrient removal efficiency. We achieved this by controlling pH in the source medium at two different HRTs, but not directly adding Mg.

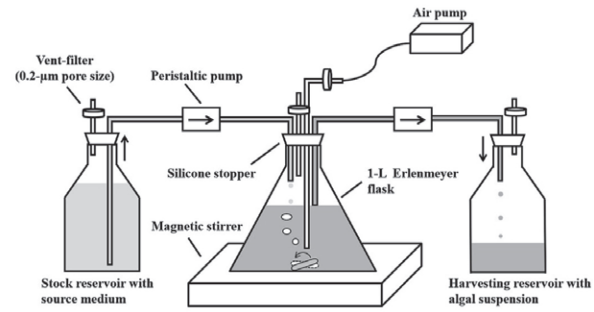

Fig. 3 Schematic diagram of experimental flow-through cultivation system.

In the continuous cultivation system adopted in this study, a pH level of 6.5 in the source medium provided suitable conditions, irrespective of HRT tested. It allowed maximum algal biomass productivity and nutrient removal from the ADE of aquatic macrophytes, by indirectly increasing the available Mg^{2+} and PO_4^{3-}-P in the culture medium. Treatment with a pH level of 7.0 was too high to provide sufficient amounts of available Mg^{2+} in the ADE for maximum algal growth, while even pH 6.0 treatments may be too low to retain dissolved inorganic carbon contents in the culture medium for maintaining photosynthesis.

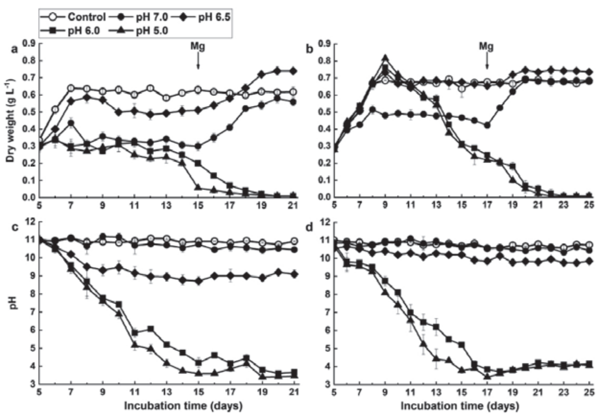

Fig. 4 Daily algal biomass concentrations (a, b) and pH levels (c, d) in culture media in control (open circles), pH 7.0 (closed circles), pH 6.5 (closed diamonds), pH 6.0 (closed squares) and pH 5.0 (closed triangles) treatments at 6 days (left panels) and 8 days (right panels) of HRT. Arrows indicate the date on which addition of Mg began.

Chapter 3

In this Chapter, *C. sorokiniana* was cultured in ADE of aquatic macrophytes treated with different durations of ultraviolet (UV) C exposure to clarify effects of UVC radiation on Mg availability for algal growth through degrading dissolved organic matter (DOM) in the ADE used due to photolysis. Then, the molecular size distribution of DOM in the ADE before and after UVC exposure were determined with a high-performance size-exclusion chromatography (HPSEC) combined with fraction collector. Finally, Mg concentrations were determined in each fraction of the DOM molecular sizes separated to clarify which sizes of DOM were associated with Mg.

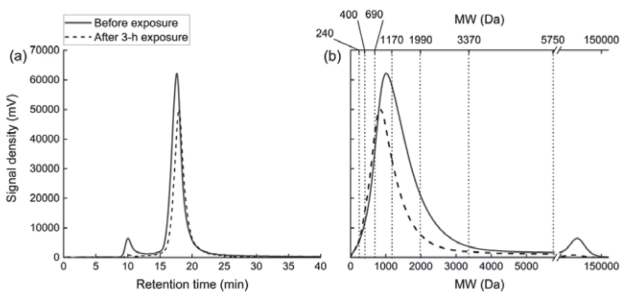

Fig. 5 HPSEC of ADEs from aquatic macrophytes before and after 3-h exposure to UVC for retention time (a) and DOM molecular weight (MW) (b). Vertical dotted lines denote six fractions of DOM molecular sizes in the ADEs for further analysis of the DOM molecular sizes adsorbed with Mg

Two peaks in DOM molecular size were found in ADE from aquatic macrophytes: 1.62×10^3 and 8.48×10^4 Da of weight-averaged molecular size. UVC exposure for 3 h decomposed DOM in the ADE, and consequently released sufficient available Mg^{2+} (0.09 mg from 1 mg DOC) to permit maximum algal yield and nutrient removal. Almost 50% of the Mg in the ADE was bound with DOM of 400–1170 Da molecular size as organic complexes, and the remaining Mg was distributed to DOM of < 240 Da and/or Mg^{2+} and inorganic Mg complexes.

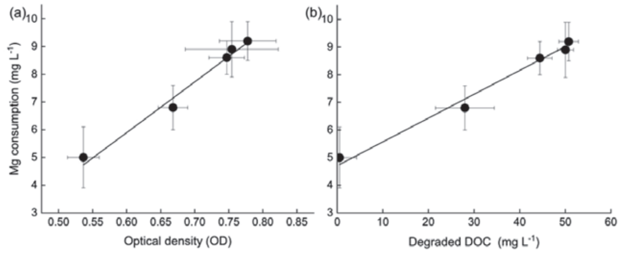

Fig. 6 Relationships between Mg consumption and algal yield as OD (a) at the end of experiments, and between Mg consumption and degraded amounts of dissolved organic carbon (DOC) (b) during algal cultivation in ADEs of aquatic macrophytes treated with and without UVC exposure.

Chapter 4

I conducted three experiments in this Chapter. 1) The shortest HRT in sequencing batch reactor (SBR) for nitrification was determined using CADE combined with submerged macrophytes and food wastes. 2) Three green algae, *C. sorokiniana*, *C. reinhardtii* and *B. braunii*, were cultured in undiluted and diluted nitrified ADEs (NADEs) from two different digestates, i.e. invasive macrophyte *L. grandiflora* (NLADE) and co-digestates (NCADE) combined with submerged macrophtes and food wastes, to determine dilution effect in each NADE for algal yield and nutrient removal. 3) Finally, *C. sorokiniana* was cultured with NCADE using a flow-through system at four different HRTs, to obtain the maximum algal biomass productivity from the NCADE.

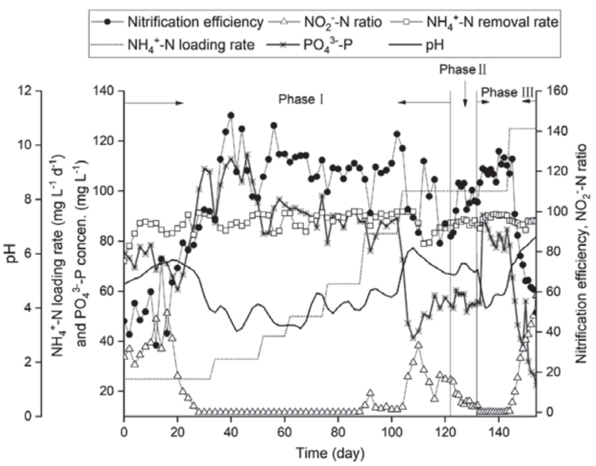

Fig. 7 Profiles of nitrification efficiency (closed circles, solid line), NO_2^--N ratio (open triangles, solid line), NH_4^+-N removal rate (open squares, solid line), NH_4^+-N loading rate (dotted line), PO_4^{3-}-P concentration (asterisks, solid line) and pH (solid line alone) as function of time in SBR.

HRT in SBR could reduce to 5 days, i.e. NH_4^+-N loading rate of 100 mg L^{-1} d^{-1}, with 18-h-aeration and 6-h-settling cycle for complete nitrification through acclimation of nitrifying sludge. Three algal species were all successfully cultured in both undiluted NCADE and NLADE contained sufficient nutrients for maximum algal yield harvesting and high PO_4^{3-}-P removal rates, but low NO_3^--N removal rates in test-tube cultivation. In continuous cultivation, 14 days of HRT was optimal for maximizing C. sorokiniana biomass productivity (110 mg L^{-1} d^{-1}), but with low nutrient removal (14% for NO_3^--N and 48% for PO_4^{3-}-P) from the undiluted NCADE probably due to low light intensity or temperature. According to the optimal HRT (i.e. 14 days) in continuous cultivation and 5 days of HRT for complete nitrification, the working volume ratio was 36%, which can be one of cost-effective design for further large-scale application.

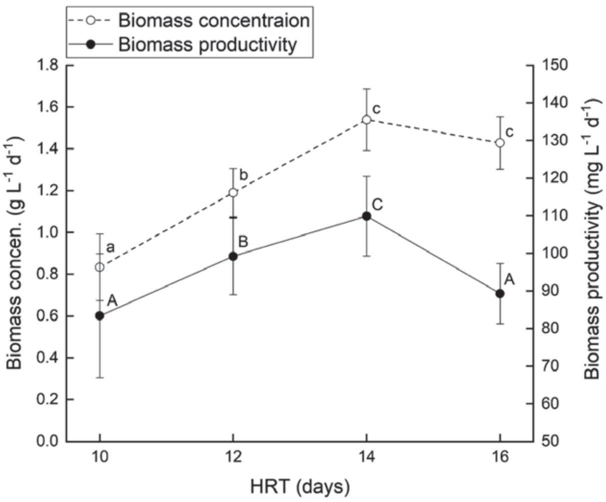

Fig. 8 Average algal biomass concentration (g L^{-1} d^{-1}) and productivity (mg L^{-1} d^{-1}) of *C. sorokiniana* in undiluted NCADE in steady running period at 10, 12, 14 and 16 days of HRT in continuous cultivation.