DISSERTATION

COUPLING PROCESS OF SIMULTANEOUS DESULFURIZATION－NITRIFICATION AND MICROALGAL CULTIVATION FOR BIOGAS AND EFFLUENT FROM ANAEROBIC DIGESTION

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We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Engineering.

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ABSTRACT

Anaerobic digestion (AD) is an economical and environmentally friendly biological treatment method of organic waste with biogas production. For further widespread of the AD, it is required to reduce environmental loads and costs for post-treatments such as desulfurization for biogas utilization and nitrification−denitrification for nitrogen removal from AD effluent (ADE). Therefore, in the present thesis, a novel coupling process of simultaneous desulfurization-nitrification (SDN) and microalgal cultivation was proposed. In the SDN, biogas desulfurization and ADE nitrification are performed in a single reactor by sulfur-oxidizing bacteria and nitrifying bacteria consortium, leading to the reduction of the number of reactors. Furthermore, because NH_4^+ becomes harmless NO_3^- via SDN, ADE treated by the SDN can be used for the production of valuable microalgae without dilution, therefore, a large amount of water consumption in the conventional microalgal cultivation would be saved. To develop the proposed process, first, the SDN treatment of biogas and ADE was established by the suppressions of sulfide inhibition to nitrification and O_2 contamination to desulfurized gas. Then, the usability of ADE treated by the SDN for microalgal cultivation was evaluated. Last, the stable coupling operation of SDN and microalgal cultivation was confirmed, and the cost/electrical energy consumption of the process was discussed.

Firstly, a sequential batch reactor (SBR), a typical aerobic reactor with high sludge retention ability, was used for the SDN treatment with a long fill period operation to suppress the sulfide inhibition to nitrifying bacteria. Stable SDN treatment was achieved at 100% efficiencies under the supply of NaHS instead of H_2S gas at up to 128 mg-S L^{-1} d^{-1} which is the highest load in the previous reports. The increase in sulfide loading rate changed dominant nitrifying bacteria and increased sulfide tolerance of NH_4^+ oxidation. The SBR with a long fill period effectively facilitated the acclimatization of a microbial consortium to sulfide. Next, synthetic biogas without CO_2 (0.5% H_2S) and ADE were treated by SDN using a continuous stirring tank membrane reactor (CSTMR) which has high sludge retention ability and continuous substrate supply such as the SBR operation and an

external O_2 bubbling column to suppress the O_2 contamination to desulfurized gas. SDN treatment of synthetic biogas and ADE was successfully operated. Also, O_2 contamination into desulfurized gas was mitigated to 0.4% on average. Desulfurized biogas is appropriate for further biogas utilization due to low explosion risk. In batch cultivation of *Chlorella sorokiniana* NIES-2173, in contrast to the decrease in the growth yield maximally by 88% when using ADE with 1–10-fold dilutions, almost the same productivity was obtained by using ADE treated by SDN, without dilution, compared with that using synthetic medium. Meanwhile, in the compositional analysis of treated ADE, it was found that treated ADE has a high salinity (0.7%) which may limit the available microalgal strain.

Throughout the above studies, the feasibility of the coupling process of SDN and microalgal cultivation for biogas and effluent from AD was assessed as being high. Last, a 3.0-L SDN reactor and a 4.5-L microalgal airlift photobioreactor (continuous light irradiation) were operated simultaneously with 3 days and 5 days of hydraulic retention time (HRT), respectively, to confirm the process stability and estimate the mass balance of sulfur, nitrogen and so on. Stable SDN treatment and high microalgal production (0.48-g L^{-1} d^{-1}) were accomplished simultaneously. Along with that, NO_3^- in SDN effluent was removed by 23% in the microalgal reactor. Therefore, nitrogen in ADE could be removed completely by SDN and microalgal reactor with a volume ratio of 3:40 (3-day and 40-day HRT) under 12h:12h of light and dark periods. The results of economic estimation of the developed process based on the mass balance data showed that high capital and annual cost for CO_2 removal from biogas and microalgal cultivation. However, since the coupling process has the potential to produce a large revenue from biomethane and microalgal biomass sales, 12-times higher net cost was estimated in the coupling process compared with conventional process (biodesulfurization of biogas and heat and power generation plus nitrification-denitrification of ADE). The development of technologies of O_2 recovery from microalgal reactor and supply to the SDN reactor to further reduce cost and energy consumption is desired as a future study for the implementation of the developed process.

Chapter 1

General introduction

1.1. Organic waste treatments and application of anaerobic digestion (AD)

Since the 20th century, the amount of waste has been rapidly increasing year by year with economic development and population growth. The estimated annual emission of municipal waste in 2016 was 2.01 billion tons, and it is predicted to be 3.4 billion tons in 2025 (Kaza et al., 2018). The United Nations Environment Programme (UNEP) and the International Solid Waste Association (ISWA) are proposing five priority Global Waste Management Goals by 2030. These goals directly linked to 12 goals in the 17 goals of the Sustainable Development Goals (SDGs) which was adopted by the Sustainable Development Summit in 2015 (Table 1-1) (Wilson et al., 2015), indicating that waste management is a crosscutting global urgent issue. The development of appropriate waste treatment systems continues to be required to establish a recycling-oriented society.

Organic waste such as food waste, livestock manure, sewage sludge, agricultural waste, and woody waste reaches 38 billion tons per year (Kiyasudeen et al., 2015), and 65% of the total amount of municipal waste (Kaza et al., 2018). Currently, organic wastes are mainly treated by landfill disposal, incineration, and compost treatment in the world. In developing countries without sufficient funds or technical know-how, the waste is primarily disposed of in the landfill. This treatment has many problems such as land shortage, the emission of greenhouse gases (CO_2 and CH_4 and toxic H_2S, etc.), and the pollution of the land and surrounding environment due to the leaching of harmful substances derived from wastes. On the other hand, the developed countries often adopt incineration of wastes. However, a high cost and the emission of CO_2 and dioxins are a problem. Although some incinerators generate power using exhaust heat, so-called “waste to energy,” the generated power is small compared to the energy saved by recycling. Thus, organic waste utilization as compost, feeds, solid fuels, methanol, fuel gas, and so on has been promoted since the 1970s. In particular, composting is world-widely applied since the 1980s when the large-scale facilities were expanded in EU countries

(Slater and Frederickson, 2001). However, recycling organic waste into these valuable materials requires matching supplies with the site's demand.

Anaerobic digestion (AD) has been attracting attention as an environmentally friendly treatment method of organic wastes due to decomposing organic carbon to biogas containing energetically usable CH_4 by anaerobic bacteria and archaea. It can handle a wide variety of organic wastes from wet solids to dry solids. Produced biogas can be used in the site and sold to the outside directly or through a power generation facility. It also can be used or sold as an alternative to natural gas after increasing CH_4 concentration by purification. Discharged digestate also can be utilized as fertilizer. Therefore, AD treatment possibly reduces environmental burdens compared with waste-to-energy incineration (Thyberg and Tonjes, 2017; Mayer et al., 2020). However, the application of AD is still only less than 0.3% for municipal solid waste treatment in the world (Kaza et al., 2018). Biogas production in 2018 was around 35 million tonnes of oil equivalent (Mtoe) which is only 6% of overall potential of biogas production from organic waste in the world (IEA, 2020). This lower percentage is because of the requirements of construction and maintenance of some post-treatment processes, in addition to challenges such as the process stability and need of pre-treatments including waste collection.

1.2. Current issues in post-treatments of the AD system

By-products of AD treatment are biogas and digestate. Biogas generated from AD generally contains 40% CO_2 and 0.05–0.3% H_2S (Soroushian et al., 2006; Alonso-Vicario et al., 2010) other than CH_4. CO_2 removal is also necessary to utilize biogas as a high calorific gas, but H_2S removal is essential regardless of the biogas applications because H_2S has high toxicity and corrosivity which causes deterioration of biogas combustion equipment. A classic and common desulfurization treatment is the physical/chemical methods using several types of adsorbents. The physical treatment, called dry-type desulfurization, generally uses iron oxide as an adsorbent (Figure 1-1, (A)-i). Besides, chemical treatments called wet-type desulfurization uses absorbents such as NaOH and alkylamines

instead. The advantages of these physical/chemical methods are the simple treatment process and easy operation. Besides, regular replacement and disposal of absorbents have cost and environmental loads. Bio-desulfurization is an alternative treatment method to oxidize H_2S mainly to SO_4^{2-} by sulfur-oxidizing bacteria. Biofiltration, a typical bio-desulfurization, performed by using fixed bed reactors under aerobic condition using O_2 as electron acceptors (Figure 1-1, (A)-ii) or anoxic condition using NO_3^-. Another type of bio-desulfurization is in-situ microaerobic H_2S oxidation by supplying limited O_2 into mainly the headspace of the AD reactor (Figure 1-1, (A)-iii). Because of less environmental load and cost and high enough H_2S removal efficiency, currently, biodesulfurization is gradually implemented in the full-scaled treatment facilities. However, environmental loads such as mechanical aeration/chemical addition and 0.013 and 0.016 € m^{-3} of operating costs were remaining for aerobic and anoxic bio-trickling filtration, respectively (Gonzalez-Sanchez et al., 2009; Fernández et al., 2014). Also, suppression of O_2 contamination into desulfurized biogas and removal of accumulated S^0 which is intermediate compounds of H_2S oxidation are remaining issues.

ADE contains a high concentration of nutrients, particularly NH_4^+ of 500–4000 mg-N L^{-1} (Walker et al., 2011; Vaneeckhaute et al., 2017) derived from decomposed organic compounds; thus, ADE can be used as a liquid fertilizer (Figure 1-1, (B)-i). However, because of the high cost to transfer it to the site, the spray area is limited, and the demand is not large enough to meet the supply (Xia and Murphy, 2016). Therefore, NH_4^+ is generally removed from ADE by a nitrification–denitrification process, i.e., oxidation to NO_3^- and reduction of NO_3^- to N_2 (Figure 1-1, (B)-ii), or other related technologies such as ANAMMOX (anaerobic ammonium oxidation) and partial nitrification, especially in an urban area. However, mechanical aeration and organic addition for the treatment involve energy consumption and cost. Also, nitrogen is missed out from land to the atmosphere. As an alternative method to recover ADE nutrients as valuable products, microalgal cultivation has recently attracted attention. Microalgae can grow only using light energy and CO_2 in addition to nutrients, and obtained biomass or its extractives are available commercially as materials for functional foods, cosmetics, and feeds (Koutra et al., 2018) (Figure 1-1, (B)-iii). Because nitrogen

can be fixed/recovered as a valuable resource, this microalgal cultivation has the potential to contribute to developing a recycling-oriented society, especially in sunbelt countries (South America, Southeast Asia, the Middle East/North Africa, etc.) where solar radiation flux and temperature are high. However, water consumption is a disadvantage of the microalgal cultivation using ADE. NH_3 is the undissociated form of ammonia and presents with NH_4^+ in ADE under neutral and alkaline pH (pKa=9.25). Because NH_3 has high toxicity for microalgal growth (Park et al., 2010; Levine et al., 2011; Collos and Harrison, 2014), ADE is usually used as a medium after 2–50 fold dilution (Olguín et al., 2003; de Godos et al., 2009; Kumar et al., 2010; Wang et al., 2010b; Park and Craggs, 2011; Lee et al., 2015; Xia and Murphy, 2016). A large amount of freshwater consumption and the disposal of this larger water volume for the dilution make it difficult to introduce the microalgal cultivation system.

Only selling of electricity derived from biogas is not enough to benefit the further application of AD on the premise that various post-treatments are necessary. Simplification and reduction of environmental loads/costs of the post-treatment process and making high-value products from by-products of AD are required.

1.3. Novel biogas and ADE treatment system with microalgal production

The present thesis proposed a novel coupling process of simultaneous desulfurization–nitrification (SDN) and microalgal cultivation for biogas desulfurization and nutrients removal from ADE, as post-treatment of AD (Figure 1-2). Both sulfur oxidation by sulfur-oxidizing bacteria and nitrification by nitrifying bacteria reacts in a similar condition: aerobic environment, neutral pH, and less than 30ºC. Therefore, these treatments may proceed in a single reactor. Also, because ADE treated by SDN contains NO_3^- which is harmless for living organisms including microalgae, the treated ADE could be used as a medium for microalgal cultivation without dilution. Furthermore, the utilization of O_2 produced by microalgae might allow less mechanical aeration in the SDN.

Several challenges are there to establish the coupling process of SDN and microalgal

cultivation. First, S^{2-} inhibits nitrification by more than 50% with a few milligrams per liter (Bejarano Ortiz et al., 2013). In the simultaneous treatment of S^{2-} and NH_4^+ using continuous stirred tank reactor (CSTR), the acclimatization of aerobic sludge to S^{2-} needed a minimum two-week inhibition period to achieve 100% nitrification efficiency after a stepwise increase of S^{2-} loading rate to 74 mg-S L^{-1} d^{-1} (Beristain-Cardoso et al., 2011). A more stable nitrification treatment is required to combine with biogas desulfurization. Second, for the direct biogas supply into the SDN reactor, evaluating the CO_2 inhibitory effect on nitrifying bacteria and oxygen contamination into biogas is necessary. That is because around 40% of CO_2 in biogas would significantly increase the dissolved inorganic carbon (DIC) concentration of the SDN reactor. Also, the suppression of O_2 contamination into biogas is necessary to avoid the explosion of biogas. Third, regarding microalgal cultivation using ADE treated by SDN as a medium without dilution, high concentrations of ADE components may inhibit microalgal growth without dilution. Moreover, concentrations of some components associated with microalgal growth may change through SDN, causing inhibition or improvement of microalgal growth. Therefore, it is required to evaluate the microalgal productivity of the treated ADE.

To establish the environmentally friendly and low-cost treatment of biogas and ADE with microalgal production, the following three studies were conducted in the present thesis to develop the proposed coupling process; (1) development of SDN process using aerobic bacterial consortium, (2) integration of biogas desulfurization and ADE nitrification, (3) evaluation of treated ADE usability for microalgal culture medium without dilution, then, implementability of coupling process of SDN and microalgal cultivation for biogas and ADE treatment was considered.

Table 1-1. Global waste management primary goals and related sustainable development goals (SDGs) (Wilson et al., 2015, modified).

Global Waste Management Primary Goals		Related references within the SDGs
By 2020	1. Ensure access for all to adequate, safe and affordable solid waste collection services	3. Good health and well-being 11. Sustainable cities and communities
	2. Eliminate uncontrolled dumping and open burning	3. Good health and well-being 6. Clean water and sanitation 11. Sustainable cities and communities 12. Responsible consumption and production 14. Life below water 15. Life on land
By 2030	3. Ensure the sustainable and environmentally sound management of all wastes, particularly hazardous wastes	7. Affordable and clean energy 12. Responsible consumption and production 13. Climate action
	4. Substantially reduce waste generation through prevention and the 3Rs (reduce, reuse, recycle) and thereby create green jobs	1. No poverty 8. Decent work and economic growth 9. Industry, innovation and infrastructure 12. Responsible consumption and production
	5. Halve global per capita food waste at the retail and consumer levels and reduce food losses in the supply chain	2. Zero hunger 12. Responsible consumption and production

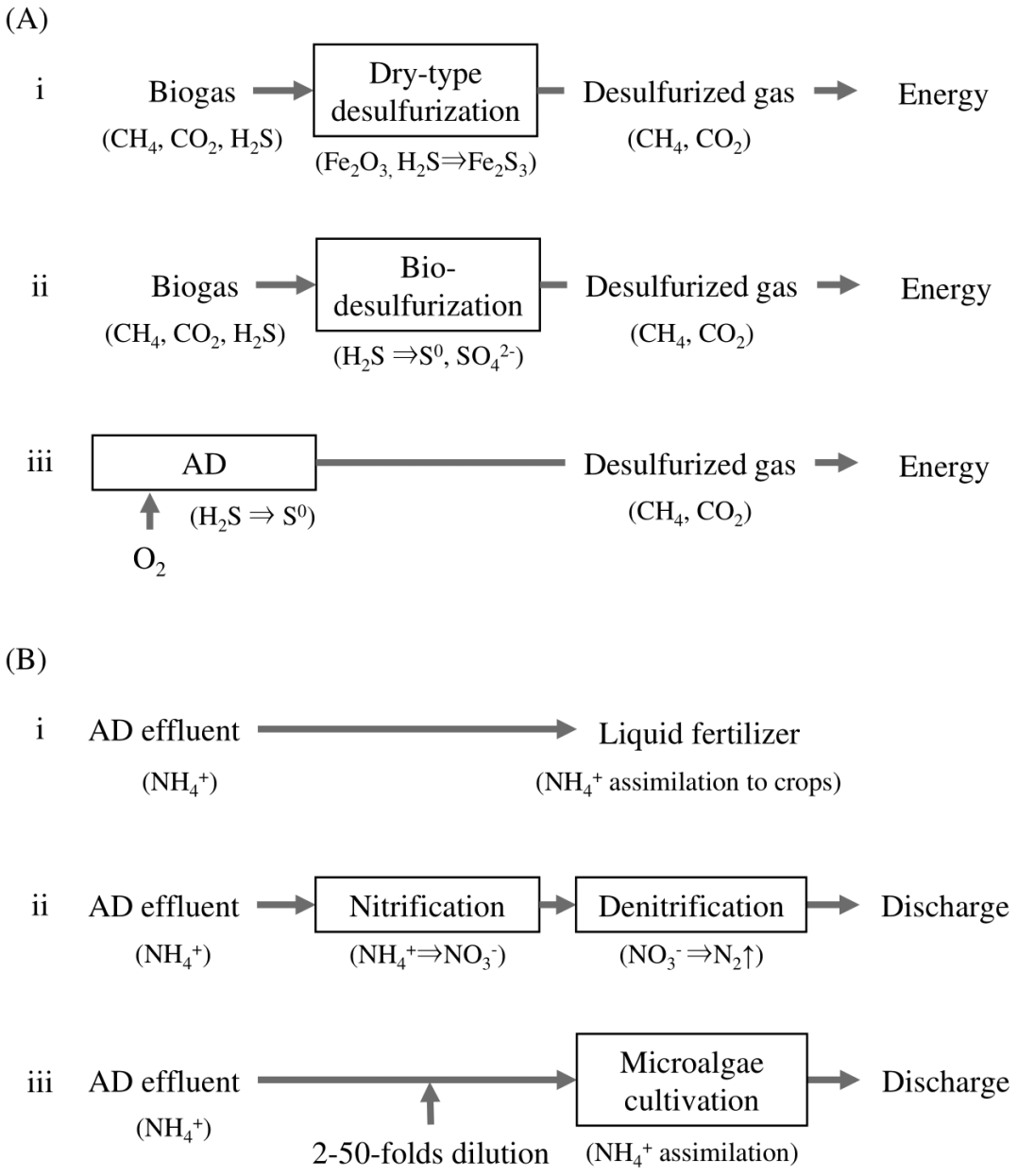


Figure 1-1. Examples of conventional post-anaerobic digestion (AD) treatments: biogas desulfurization (A) and NH_4^+ removal from AD effluent (B).

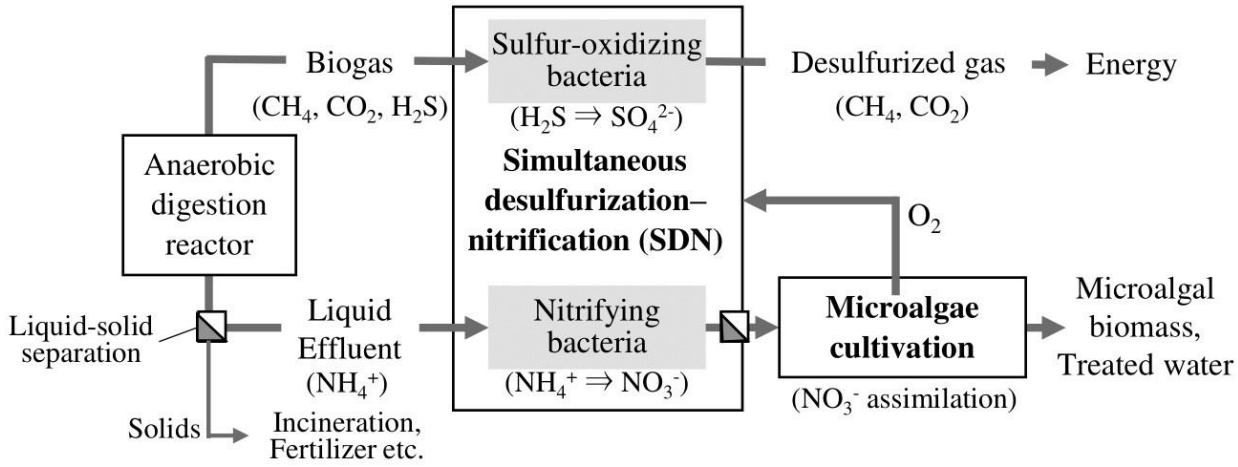


Figure 1-2. A coupling process of simultaneous desulfurization–nitrification and microalgae cultivation for biogas desulfurization and digested liquid effluent treatment (Japanese Patent Application No. 2019-35294)

Chapter 2

Simultaneous desulfurization–nitrification (SDN) using aerobic bacterial consortium

2.1. Introduction

The anaerobic digestion (AD) treatment of organic wastes produces biogas and effluent containing toxic and corrosive H_2S and high concentrations of nutrients, respectively, as byproducts. Therefore, biogas desulfurization and nitrification–denitrification of AD effluent (ADE) are often introduced as post-AD treatments. The integration of these treatments has been attempted since the 1990s to reduce the treatment reactor, energy consumption, and cost. In particular, the coupling of desulfurization and denitrification in anoxic condition has been studied (Figure 2-1(A)) (Kleerebezem and Mendez, 2002; Beristain-Cardoso et al., 2009; Deng et al., 2009; Li et al., 2009; Fernández et al., 2014). Meanwhile, desulfurization using excess O_2 in an aerobic reactor has also been studied. Nishimura and Yoda (1997) reported that 960 m^3 d^{-1} of biogas with 300–2500-ppm H_2S was treated with 550 m^3 d^{-1} of ADE derived from potato processing wastewater, simultaneously, using an aeration tank with a biogas-broth contact tower, and a 99% H_2S removal efficiency was achieved (Figure 2-1(B)-i). Also, recently, a novel coupling process of biogas desulfurization and ADE aerobic treatment was developed using a microbial consortium mainly composed of nitrifying bacteria, sulfur-oxidizing bacteria and microalgae (Bahr et al., 2014; Lebrero et al., 2016; Posadas et al., 2017) (Figure 2-1(B)-ii). While desulfurization with denitrification is better to avoid oxygen contamination into purified biogas (Xiao et al., 2014), the development of simultaneous desulfurization and nitrification (SDN), in which nitrogen remains in the ADE, is required for post-utilization of nutrients in ADE for crops and microalgae (Botheju et al., 2010; Fuchs and Drosg, 2013).

To develop SDN treatment of biogas and ADE, a decrease in nitrification efficiency under a high S^{2-} loading rate is a severe problem since S^{2-} is a strong inhibitor of nitrifying bacteria. In batch bioassay, the NH_4^+ removal rate of nitrifying sludge decreased by 51–92% by S^{2-} addition at 3.1–112.0 mg-S L^{-1} (Bejarano Ortiz et al., 2013). In the experiment using sequencing batch reactor (SBR)

with 0.57-d hydraulic retention time (HRT) and 68-d sludge retention time (SRT), almost 100% of nitrification efficiency was maintained under S^{2-} loads up to 17.5 mg-S L^{-1} d^{-1}; however, after increasing to 26.3 mg-S L^{-1} d^{-1}, it decreased by 90% and NO_2^- was accumulated for 50 days. After recovering the efficiency, nitrification efficiency dropped again until 0% by increasing sulfide loading rate (SLR) to 35 mg-S L^{-1} d^{-1}. Then, it gradually recovered over 210 days (Bejarano Ortiz et al., 2020). In a continuous treatment of synthetic wastewater containing NH_4^+ and S^{2-} using a continuous stirred tank reactor (CSTR) (Beristain-Cardoso et al., 2011), 100% of NH_4^+ removal efficiency was achieved under 32- and 76-mg L^{-1} d^{-1} S^{2-} loading rate and 1.8 d HRT. However, a two-week inhibition period was required before stabilizing treatment efficiency at each S^{2-} loading rate. In a previous study using SBR with a long fill period, complete nitrification was achieved under 32 mg-S L^{-1} d^{-1} SLR just after two days inhibition period (Sekine et al., 2018). SBR is a general reactor type known to retain sludge effectively than CSTR since its operation consists of fill, react, settle and discharge periods and the only supernatant is discharged as effluent. This higher sludge retention time (SRT) probably prevented the wash-out of slow-growing microbes including nitrifying bacteria. Moreover, in SBR operation with a long fill period, the substrate is gradually supplied over the whole reaction period instead of the usual short term supply before the reaction period. This slower supply of substrate contributed to avoid a sudden increase in S^{2-} concentration in the reactor. Therefore, this SBR with long fill period which has high SRT and slow substrate supply has a potential to acclimatize microbes effectively to S^{2-} and to maintain high nitrification efficiency under high SLR. This chapter aims to clarify the maximum SLR capacity of the SDN by SBR with a long fill period operation.

2.2.Materials and Methods

2.2.1. Substrate and inoculum

ADE and NaHS solution instead of H_2S gas were used as substrate. ADE was obtained from a mesophilic AD reactor treating sewage sludge, after centrifugation for liquid–solid separation with polyferric sulfate in Hokubu Sludge Treatment Center, Yokohama, Japan. The obtained ADE was

stored in a freezer at -30ºC, and melted and filtered with grass-fiber filters (0.45 μm pore size; GC-50; Advantec) before usage. Then ADE and NaHS solution were stored separately in a refrigerator at 4ºC near the reactor and supplied together to the treatment reactor by each pump (Figure 2-2). ADE and NaHS solution in a refrigerator were replaced every three days.

As inoculum, nitrifying sludge treating ADE in the same center in Yokohama, Japan was obtained. The sludge was used after being sieved through at 500-μm wire mesh, and washed with distilled water to remove any dissolved compounds.

2.2.2. Reactor operation

A 3.0-L cylindrical SBR was used with 2.1 L effective volume (Figure 2-2). The reactor was operated on a 24 h cycle consisted of 23.5 h filling and reaction phase, 20 min sedimentation phase, and 10 min decanting phase. The reactor was agitated at 200 rpm by a magnetic stirrer. The temperature was maintained at 30ºC by using a water bath. During the filling and reaction phase, pH and dissolved oxygen (DO) concentration were adjusted to 7.5 and within 1.5–4.0 mg-O_2 L^{-1}, respectively, by automatic addition of 2 N NaOH using a process controller (EYALA EPC-2000; Tokyo Rika, Japan) and continuous aeration using an air pump and a flow control valve. HRT was regulated for three days, resulting in the 293±35 mg-N L^{-1} d^{-1} of NH_4^+ loading rate (NLR). SLR was increased stepwise from 0 to 32, 64, 128, and 256 mg-S L^{-1} d^{-1} by changing NaHS solution concentration from 0 to 768 mg-S $L^{-}ADE^{-1}$. Each SLR period is referred to as Phase 1–5 (Figure 2-3).

2.2.3. Analytical parameters

Regarding the exhausted gas, H_2S concentration was measured once per 3 days using a gas detection tube which has a 0.25–120 ppm measuring range (4LL; Gastec, Japan) after collecting gas in an aluminum gas bag during the filling and reaction phase. Regarding the liquid phase, the NH_4^+, NO_2^-, NO_3^-, $S_2O_3^-$ and SO_4^{2-} concentrations of the influent and effluent were analyzed using high-performance liquid chromatography (HPLC) with an electrochemical detector (CD-5; Showa Denko, Japan) and two columns (IC YS-50 for cation and IC I-524A for anion; Showa Denko, Japan). The

S^{2-} concentration of effluent was determined by the methylene blue spectrophotometric method in reference to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The dissolved organic carbon (DOC) concentration was measured by the catalytic oxidation method (TOC-L_{CPN}; Shimadzu, Japan). As an indicator of microbial concentration, volatile suspended solids (VSS) concentrations of the reactor inside and effluent were determined according to the sewage analytical methods of the Japan Sewage Works Association (JSWA, 1997).

Based on the VSS concentration, the SRT was calculated as follows:

$$SRT\ (day) = (V \times X_r) / (Q_e \times X_e) \quad (2\text{-}1)$$

where V [L] is the effective volume of the reactor, X_r and X_e [g-VSS L^{-1}] are the VSS concentration of the reactor inside and effluent, respectively, and Q_e [L d^{-1}] is the effluent volume in a day (0.7 L d^{-1}) in this experiment.

2.2.4. Bioassays of aerobic sludge

To evaluate the S^{2-} tolerance of NH_4^+ removal ability and S^{2-} removal rate of the SBR sludge, a bioassay was conducted under different S^{2-} concentration in the range of 0–64 mg-S L^{-1} at the end of each phase from 1 to 4 in reference to Bejarano-Ortiz et al. (2013). 0.45-μm filtered ADE was used as a substrate with 20 times dilution and 1.5-g-C L^{-1} $NaHCO_3$ addition as a pH buffer; thus, NH_4^+ concentration was approximately 40 mg-N L^{-1}. pH was adjusted to 7.5±0.05 by HCl addition. Serum bottles of 160 mL were used as containers with 100 mL of effective volume: 94.5 mL of the above ADE substrate, 0.5 mL NaHS solution plus 5 mL of the SBR sludge concentrated by settling and decantation, resulting in that initial SS concentration was adjusted to 0.2 g-VSS L^{-1}. Abiotic control used ultrapure water instead of the sludge was also prepared. The sludge and NaHS solution was added into the bottle after bubbling pure O_2 gas in the substrate for 3 minutes, and then, the bottle was immediately closed with a rubber cap and an aluminum seal. The assay was conducted for 24 hours under 30±1°C temperature and 160 rpm agitation using a thermostat shaker. All assays were performed in triplicate.

During the assays, the NH_4^+ concentration in all conditions and S^{2-} concentration in 64 mg-S

L^{-1} condition were periodically measured using the same analytical methods written in section 2.2.3. After the assays, the DO concentration and pH were measured using a DO probe (InLab 605; Mettler Toledo, USA) and a desktop pH meter (SevenCompact pH/Ion meter S220; Mettler Toledo, USA), respectively.

Based on the results of NH_4^+ and S^{2-} removal rates, the 50% inhibitory S^{2-} concentration (S^{2-}-IC_{50}) for NH_4^+ removal efficiency, which is an indicator of the S^{2-} tolerance of the sludge was evaluated as follows (Zhou et al., 2014):

$$AUR = AUR_0 \times K_i / (K_i + S_i) \times 100 \quad (2\text{-}2)$$

where the AUR and AUR_0 [g-N g-VSS^{-1} h^{-1}] are the NH_4^+ uptake (removal) rate in a certain initial S^{2-} concentration and zero concentration conditions, respectively. S_i [g-S L^{-1}] is the initial S^{2-} concentration. K_i [mg-S L^{-1}] is the half-saturation coefficient which is the same with S^{2-}-IC_{50} for NH_4^+ removal efficiency. AUR was obtained by linear regression of the time-course change of NH_4^+ concentration until before running out of NH_4^+. The maximum S^{2-} removal rate was estimated by applying the integrated Gompertz model to the time-course change of S^{2-} concentration in 64 mg-S L^{-1} initial S^{2-} concentration condition in reference to Acuna et al., (1999) and Draper and Smith (1981) as follows:

$$S_c = \alpha \exp(-\beta e^{-Kt}) \quad (2\text{-}3)$$

$$V_{max} = 0.368\alpha K \quad (2\text{-}4)$$

where S_c [mg-S L^{-1}] is the consumed S^{2-} concentration, α [mg-S L^{-1}] is the maximum S^{2-} concentration consumed, that is, the initial S^{2-} concentration in this assays, β is a parameter related to the initial conditions ($S=S^0=\alpha\exp(-\beta)$ at t=0), K [h^{-1}] it the S^{2-} consumption rate, and V_{max} [mg-S L^{-1}] is the maximum S^{2-} consumption (removal) rate.

2.2.5. Bacterial community analysis

At the end of each SLR phase, SBR sludge was collected and stored in the freezer at -30ºC until just before DNA extraction. The extraction of total DNA from samples was conducted by an Extrap Soil DNA Kit Plus ver. 2 (Nittetsu Sumikin Kankyo, Japan). Then, the bacterial 16S rRNA

gene V4 region (250 bp) was amplified by the first polymerase chain reaction (PCR) using the primers 515F (50-GTGCCAGCMGCCGCGGTAA) and 806R (50-GGAC-TACHVGGGTWTCTAAT), and indexed by the second PCR using the Nextera XT index primers (Illumina, USA). After the PCR, obtained 16S rRNA clone libraries were purified using the AMpure Beads XP (A63880; Beckman Coulter, USA). After that, the PCR amplicons were quantified for concentration with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). These were sequenced by an Illumina MiSeq using the MiSeq Reagent Kit v2 after adding the PhiX control library. All the useful sequences were analyzed in the Quantitative Insights Into Microbial Ecology 2 software package (2018.6 release). These were grouped into corresponding taxonomies differing by as little as one nucleotide and referred to as amplicon sequence variants (ASVs) (Callahan et al., 2017) using Deblur. Each ASV was classified using a 99% OTU reference 16S rRNA database of Greengenes 13_8 (greengenes.lbl.gov).

2.3. Results and Discussion

2.3.1. S^{2-} and NH_4^+ treatment efficiency

Throughout the experimental period, S^{2-} in the effluent (Figure 2-4, (A)) and H_2S gas in exhausted gas (Data was not shown) were not detected. SO_4^{2-} concentration of effluent increased gradually up to 200 mg-S L^{-1} with an increase in SLR in Phase 1–3. Besides, in Phase 4–5, the concentration was not changed from Phase 3 except for the last 15 days of Phase 5. White precipitants attaching to the reactor was observed at the end of the experiment. S^{2-} often precipitates as S^0 produced in the process of S^{2-} oxidation (Pokorna and Zabranska, 2015). Therefore, complete removal of the supplied S^{2-} was mainly by oxidation to SO_4^{2-} and also possible by partial oxidation to S^0. S^0 is harmless to microbes including nitrifying bacteria (Nguyen, 2017), but it must periodically pull out the acclimated S^0 from the reactor in continuous treatment.

Complete nitrification was achieved without S^{2-} inhibition period in Phase 1–4 with 0–128 mg-S L^{-1} d^{-1} of SLR (Figure 2-4, (B)). NH_4^+ and NO_2^- concentrations of effluent were below the

detection limit and NO_3^- was produced with the almost same concentration with supplied NH_4^+. Besides, in Phase 5 with 256-mg-S L^{-1} d^{-1} SLR, the NO_3^- concentration of effluent dropped after day 97, and NH_4^+ was acclimated. And then, almost NH_4^+ was partially oxidized to NO_2^- continuously. Similarly, VSS concentration indicating the number of microbes in the reactor also decreased just after the starting of Phase 5 from 2.0±0.3 g-VSS L^{-1} (average of Phase 1–4) to less than 0.12 g-VSS L^{-1} in the first two weeks of Phase 5. As a reason for the decrease in VSS in the SBR, (i) low growth rate, (ii) high decay rate, and (iii) deflocculation and wash out of nitrifying sludge are generally expected (Larsen et al., 2008; Sheng et al., 2010). In this experiment, the amount of VSS washed out as effluent is less than 20% of lost VSS in Phase 5 (Figure 2-5). Therefore, an increase in a microbial decay rate exceeding the growth rate by S^{2-} inhibition was probably the main reason for the decrease in the number of microbes may including the NO_2^- oxidation bacteria inside the reactor, causing the accumulation of NO_2^-.

2.3.2. S^{2-} tolerance and removal rate of SBR sludge in each S^{2-} loading rate phase

The acclimatization of microbial communities to inhibitors is generally carried out by acquiring resistance to the inhibitor at the individual cellular and/or community level (Bhakta, 2016). The increased removal rate of target toxic compounds in the reactor is another possible acclimatization mechanism since the toxicity intensity often depends on the exposure duration to the inhibitors (Vaiopoulou and Gikas, 2012). To understand these acclimatization mechanisms that facilitate establishing microbial communities with high sulfide tolerance, the batch bioassay was conducted using the SBR sludge taken at the end of each phase. After 24 h batch bioassays, the DO concentration was more than 15 mg-O_2 L^{-1} and pH was between 7.23 and 7.92 in every condition. During the experiment, the NH_4^+ concentration decreased linearly with time except for 64 mg-S L^{-1} concentration conditions in Phase 2 and 4 (Figure 2-6). The NH_4^+ removal rate tended to be higher in conditions at lower initial S^{2-} concentration and using SBR sludge under high SLR. The S^{2-}-IC_{50} of NH_4^+ removal ability calculated based on the NH_4^+ removal rate was 1.43, 3.65, 2.77, and 5.82 mg-S L^{-1} in Phase 1–4, respectively, and increased four times from Phase 1 to Phase 4 (Figure 2-7, Table 2-

1). Besides, the maximum S^{2-} removal rates estimated from the time course of S^{2-} concentration did not increased but rather tend to decrease from 0.31 to 0.17 g-S g-VSS^{-1} h^{-1} in the latter phases (Figure 2-8, Table 2-1). Therefore, sulfur-oxidizing bacterial activity was not the reason for the increase in IC_{50}. Instead of that, the resistance of the nitrifying bacteria may have increased throughout the SBR operation by expressing an inhibition-suppression mechanism in each cell or by a change in the community structure.

In the previous studies, S^{2-}-IC_{50} values for NH_4^+ oxidizing ability of nitrifying sludge have been reported in a wide range from 0.73 to 13 mg-S L^{-1} (Table 2-2). Nitrifying sludge usually contains sulfur-oxidizing bacteria, therefore, S^{2-}-IC_{50} must have also been affected by the sludge concentration in addition to the activity of sulfur-oxidizing bacteria in used sludge. S^{2-}-IC_{50} values in the previous studies and present study (Phase 1) tended to be high when biomass concentration of inoculum was high (Figure 2-9), thereby suggesting that the IC_{50} value for unit biomass concentration may be more useful than the normal IC_{50} value for comparing different experimental results. As a result of the division of the IC_{50} by biomass concentration (Table 2-2), the IC_{50} of the SBR sludge in Phase 1 was within the range of reported values, and the sludge in Phase 4 which is after acclimatization to S^{2-} showed the highest IC_{50} value as expected.

2.3.3. Bacterial community dynamics

The numbers of high-quality reads obtained from each of the four sludge samples taken at the end of each phase were 50,943–88,760. The observed ASVs were classified to phylum-, class-, order-, family-, genus-, and species-level with 96.8–100.0%, 95.2–99.7%, 92.5–97.2%, 72.2–88.6%, 40.5–63.4%, and 10.9–25.2% of identification rates, respectively. In the classification (Figure 2-10), two ammonia-oxidizing bacteria, were detected, *Nitrosomonas europaea* and *Nitrosomonas nitrosa*. *N. europaea* and *N. nitrosa*, which belong to the same genus-group, were dominant in the different phases: in Phase 1 without S^{2-} supply and in phases 2–4 under an SLR of 32–128 mg-S L^{-1} d^{-1}, respectively. *Nitrosomonas* was reported as a genus having higher S^{2-} tolerance than *Nitrosospira*, which is also a major ammonia-oxidizing bacterium (Delgado Vela et al., 2018). Also, *N. europaea*

has been used as an inoculum for nitrification reactors (Chung and Huang, 1998; Uemoto et al., 2000), even for the simultaneous treatment of NH_4^+ and S^{2-} (Chung et al., 2007). However, this shift in dominant species with the increase in SLR suggests that the S^{2-} tolerance of nitrifying bacteria can differ at the species level, and *N. nitrosa* might have higher S^{2-} tolerance than *N. europaea*. In nitrite-oxidizing bacteria, *Nitrospira* spp. and *Nitrobacter* spp. dominated in phases 2–3 and phase 4, respectively. While *Nitrobacter* is an r-strategist, *Nitrospira* is known to be a K-strategist which is easier to grow in a continuous treatment reactor (Nogueira and Melo, 2006). For this reason, *Nitrospira* may have grown well under lower SLR. Meanwhile, it has been suggested that *Nitrospira* has a lower tolerance to inhibitory substances than does *Nitrobacter* because *Nitrospira* has the nitrite oxidoreductase enzyme (Nxr) in periplasm while *Nitrobacter* has the enzyme in the cytoplasm (Nowka et al., 2015; Delgado Vela et al., 2018). In practice, a lower activity of *Nitrospira* than those of *Nitrobacter* and *Nitrotoga* was shown under S^{2-} supply (Delgado Vela et al., 2018). Thus, *Nitrobacter* may have been more competitive than *Nitrospira* in phase 4 under high SLR. These results show that the compositional shift in the nitrifying bacterial community contributed to maintaining stable nitrification efficiency under an SLR of 0–128 mg-S L^{-1} d^{-1}. The three genera of sulfur-oxidizing bacteria, *Paracoccus*, *Thiobacillus*, and *Hyphomicrobium,* dominated. In particular, *Hyphomicrobium* increased from 0.4% to 30.4% of the relative abundance throughout the experiment. *Hyphomicrobium* is a chemolithoheterotroph and grows optimally at neutral pH and mesophilic temperature (Gliesche et al., 2005), which is the same as the SBR operating conditions in the present experiment (pH 7.5 and 30ºC). This genus was also reported to oxidize H_2S mainly to S^0 and accumulate the S^0 inside its cells until the further oxidization of S^0 to sulfate (Chung et al., 1997). This characteristic of this genus was probably the reason of incomplete S^{2-} oxidation to SO_4^{2-} in the SBR. DOC concentration of effluent slightly increased from 60 mg L^{-1} to 100 mg L^{-1} throughout the SBR operation (Figure 2-11). It can be derived from ADE and possibly dead cells under sulfide inhibition. Because *Paracoccus* decreasing through the SBR operation is a facultative autotroph bacterium, the presence of DOC or dead cells, may have changed the sulfur-oxidizing bacterial

community from an autotrophic bacterium (*Paracoccus*) to a heterotroph bacterium (*Hyphomicrobium*). The growth of other heterotrophic bacteria such as *Rhodobacter* might also have been caused by consuming these organic carbons. While *Rhodobacter* is well-known as an anaerobic sulfur-oxidizing bacterium, it is possible that *Rhodobacter* contributed to sulfide oxidation because it has been reported to be a sulfur-oxidizing enzyme (sulfide-quinone reductase) (Schütz et al., 1998). However, although the abundance of *Hyphomicrobium* increased to 30%, the result of batch bioassay did not show an increase in S^{2-} removal rate of sludge (Table 2-1; Figure 2-8). The sulfide oxidation rate of the grown *Hyphomicrobium* spp. might have been lower than that of *Paracoccus* spp., or perhaps the *Hyphomicrobium* grew by using energy derived more from the decomposition of organic carbon than from sulfide oxidation.

2.3.4. Comparison of reactor operation with previous studies

Table 2-3 shows the efficiencies of nitrification and desulfurization in previous studies and the present study. The reactor types differed among the experiments: a fluidized bed reactor (Æsøy et al., 1998), a CSTR (Beristain-Cardoso et al., 2011), a general SBR (Erguder et al., 2008; Bejarano Ortiz et al., 2020), and the SBR with a long fill period in our previous study (Sekine et al., 2018) and the present study. All previous studies conducted simultaneous nitrification and desulfurization with lower SLRs than 80 mg-S L^{-1} d^{-1}. Nitrification efficiency was achieved 100% in a CSTR, a general SBR, and the long fill period SBR in the previous studies and present study. Furthermore, in contrast to CSTR and a general SBR operation, which caused unstable nitrification (i.e. the acclimatization period of microbes to S^{2-}) for two weeks and six weeks, respectively, the SBR with a long fill period maintained a nitrification efficiency at almost 100% throughout the operational period under high SLR of 128 mg-S L^{-1} d^{-1}. As mentioned previously, the combination of high sludge retention ability and gradual substrate supply probably promoted nitrifying bacterial acclimatization to S^{2-} and maintained high nitrification efficiency.

As examples of biogas production during AD treatment, food waste generally containing 0.17–0.26 kg VS L^{-1} of organic matter (Zhang et al., 2014) can be converted to 200–450 L of biogas

per 1.0 kg VS (Gunaseelan, 1997). Discharged ADE is usually separated into solid of 10–20%vol and liquid of 80–90%vol fractions by means of a centrifuge or screw press (Fuchs and Drosg, 2013; Xia and Murphy, 2016). Based on these biogas and ADE production in the AD, the volumetric ratio of biogas-to-ADE is calculated to be 38–130. Assuming a 70 of biogas-to-ADE ratio, 500–3000 ppm of a biogas H_2S concentration (Soroushian et al., 2006; Alonso-Vicario et al., 2010), and a 3.0 day of HRT, the SLR reaches 15–100 mg-S L^{-1} d^{-1} in the SDN treatment of biogas and ADE after AD treatment. This SLR range is less than 128 mg-S L^{-1} d^{-1} treated stably in the present study. Therefore, the present study demonstrated the possibility of applying stable SDN treatment to an actual AD plant.

Table 2-1. NH_4^+ oxidation 50 % inhibitory S^{2-} concentration (S^{2-}-IC_{50}) and maximum S^{2-} removal rate of the sludge taken from the reactor at the end of each phase.

Phase	S^{2-}-IC_{50} (mg-S L^{-1})	Maximum S^{2-} removal rate (g-S g-VSS^{-1} h^{-1})
1	1.43	0.31
2	3.65	0.25
3	2.77	0.14
4	5.82	0.17

Table 2-2. 50% inhibitory S^{2-} concentration (S^{2-}-IC_{50}) for NH_4^+ oxidation rate of nitrifying sludge (or pure culture of *Nitrosomonas europaea*) in the previous literatures and the present study.

Inoculum sampling site			Biomass (g-VSS L^{-1})	S^{2-}-IC_{50} for NH_4^+ oxidation rate (mg-S L^{-1})	IC_{50} per biomass conc.* (mg-S $gVSS^{-1}$)	References
System	Substrate	Loading rate (mg L^{-1} d^{-1})				
Aerobic reactor in the A_2O process	Anaerobic digested effluent	—	Approximately 1.3	12	9.2	[1]
CSTR (HRT 3.5 d)	Synthetic medium	NH_4^+-N: 50	0.32	2.6	8.1	[2]
The extended aeration process	Municipal wastewater	—	Approximately 2.3	9.5	4.1	[1]
Fluidized bed reactor (HRT 0.9 d)	Synthetic medium	NH_4^+-N: 220 $S_2O_3^{2-}$-S: 55	4.0	13	3.3	[3]
SBR with a long fill period (HRT 3.0 d)	Anaerobic digested effluent	NH_4^+-N: 330	0.23	0.73	3.2	[4]
Aerobic reactor in the A_2O process	Anaerobic digested effluent	NH_4^+-N: — S^{2-}-S: 3.56 (mg L^{-1}, Concentration)	2.7-3.0	>6.2	—	[5]
Pure culture of *Nitrosomonas europaea*	Synthetic medium	—	0.20	<3.2	—	[6]
CSTR (HRT 3.5 d)	Synthetic medium	NH_4^+-N: 50	—	2.54	—	[7]
Lab. Scale reactor	Synthetic medium	—	0.15	>1, <5	—	[8]
SBR with a long fill period (HRT 3.0 d)	Anaerobic digested effluent	NH_4^+-N: 293 S^{2-}-S: (1) 0 (2) 32 (3) 64 (4) 128	0.20	(1) 1.43 (2) 3.65 (3) 2.77 (4) 5.82	(1) 7.2 (2) 18.2 (3) 13.9 (4) 29.1	Present study

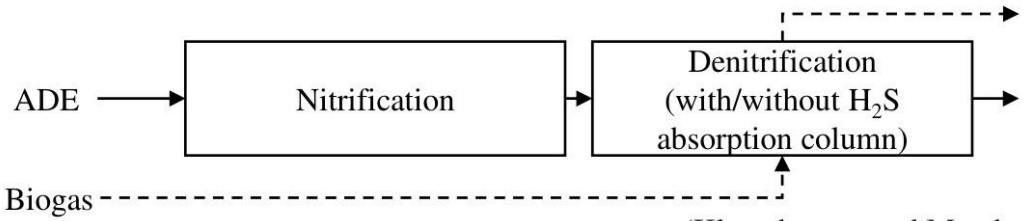
[1] Delgado Vela et al. (2018); [2] Bejarano-Ortiz et al. (2013); [3] Beristain-Cardoso et al. (2010); [4] Sekine et al. (2018); [5] Zhou et al. (2013); [6] Hooper and Terry (1973); [7] Bejarano-Ortiz et al. (2015); [8] Beccari et al.(1980). CSTR: Continuous stirred tank reactor; A_2O process: Anaerobic–Anoxic–Aerobic process; SBR: Sequential batch reactor. *: Calculated based on reported volatile suspended solids (VSS) concentration and IC_{50} values.

Table 2-3. Comparison of continuous nitrification performance under S^{2-} supply in the previous studies and the present study. Nitrifying sludge was used as inoculum in every study.

Reactor	Hydraulic retention time	Loading rate (mg L^{-1} d^{-1})		NH_4^+-N oxidation rate (mg L^{-1} d^{-1})	NH_4^+-N oxidation efficiency (%)	References
		NH_4^+-N	S^{2-}-S			
Fluidized bed reactor	1-2 h	About 400	0-60	0-148	0-37	[1]
SBR	3.0 d	230	1.3-80	92	40	[2]
SBR	0.57 d	219	0-35	219 (after 6 weeks, with NO_2^- accumulation)	100 (after 6 weeks, with NO_2^- accumulation)	[3]
CSTR	1.8 d	220	0-76	220 (after 2 weeks)	100 (after 2 weeks)	[4]
SBR with a long fill-period	3.0 d	350	32	350 (after 2 days)	100 (after 2 days)	[5]
SBR with a long fill-period	3.0 d	293	0-128	293	100	Present study

[1] Æsøy et al. (1998); [2] Erguder et al. (2008); [3] Bejarano Ortiz et al. (2020); [4] Beristain-Cardoso et al. (2011); [5] Sekine et al. (2018). SBR: Sequential batch reactor; CSTR: Continuous stirred tank reactor

(A) Conventional coupling process of desulfurization and denitrification (Anoxic)

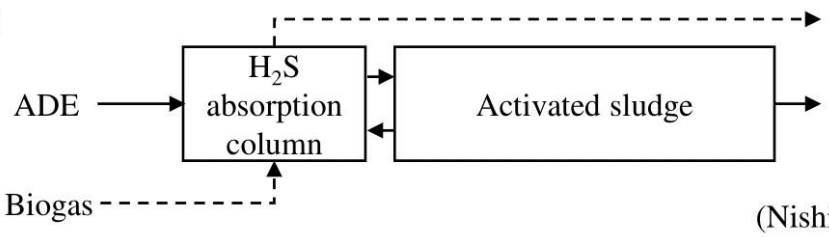


(Kleerebezem and Mendez, 2002)

- Easy to avoid O_2 contamination into biogas
- Nitrogen is missed out to the atmosphere

(B) Conventional coupling process of desulfurization and aerobic ADE treatment

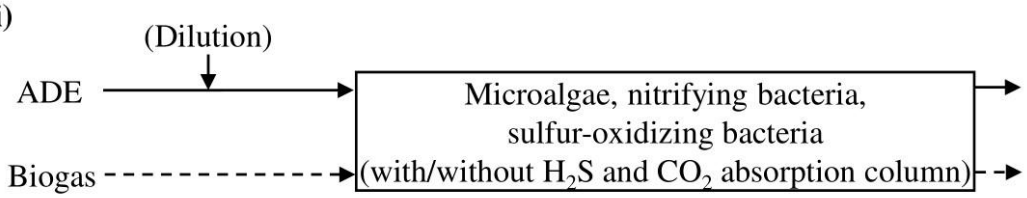
(i)



(Nishimura and Yoda, 1997)

- Excess O_2 for activated sludge can be utilized for desulfurization
- Effective NH_4^+ removal from ADE is out of focus

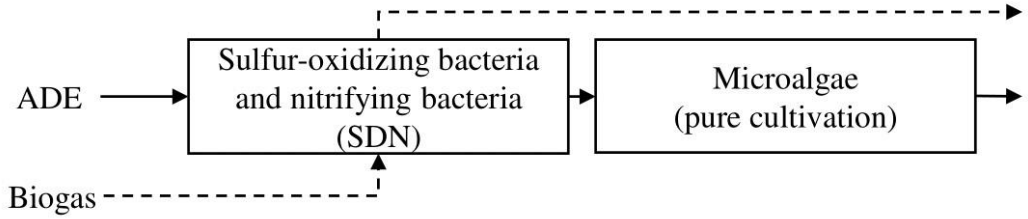
(ii)



(Bahr et al., 2014; Lebrero et al., 2015; Posadas et al., 2017)

- O_2 produced by microalgae can be used for desulfurization/nitrification
- The number of reactor is less
- Using ADE after dilution is preferable to completely avoid NH_3 inhibition
- Bacterial contamination limits the utilization of microalgae

(C) The present study



- Produced microalgae can be used for high- added-value product without dilution
- O_2 produced by microalgae is hopefully used for desulfurization/nitrification
- Inhibition of nitrification by relatively higher sulfide loading rate is concerned

Figure 2-1. Examples of simultaneous treatments of biogas desulfurization and nutrient removal from anaerobic digestate (AD) proposed in the previous studies and the present study.

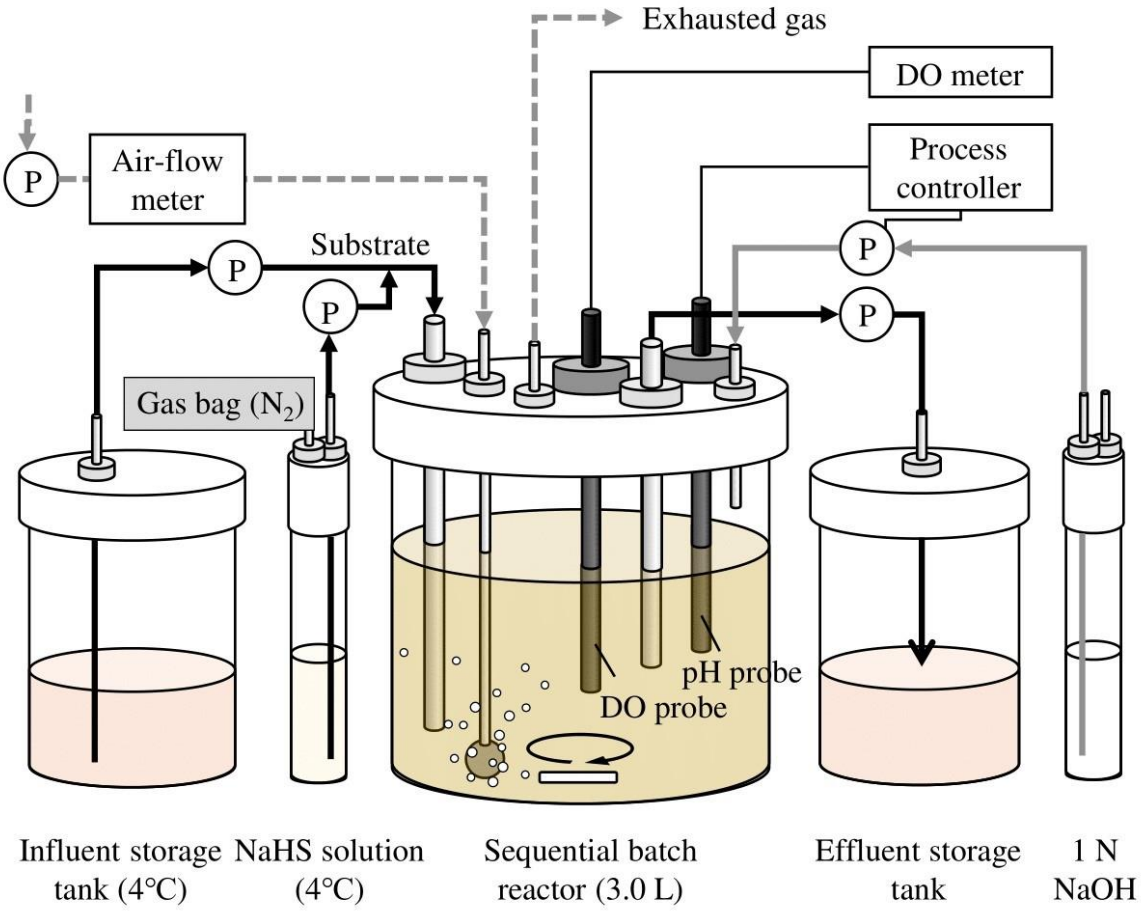


Figure 2-2. Schematic diagram of a sequential batch reactor used in the present study.

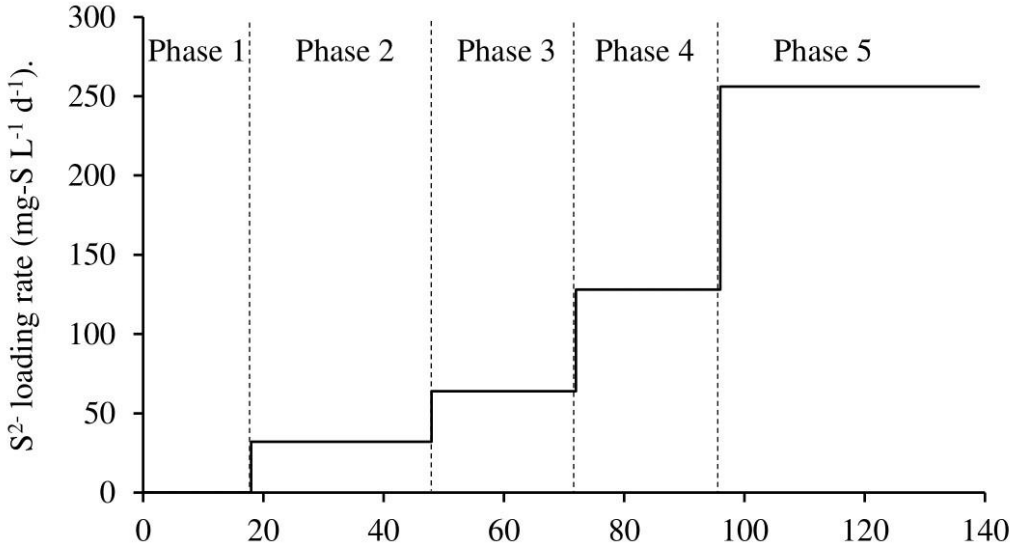


Figure 2-3. Operational schedule of S^{2-} supply in the reactor.

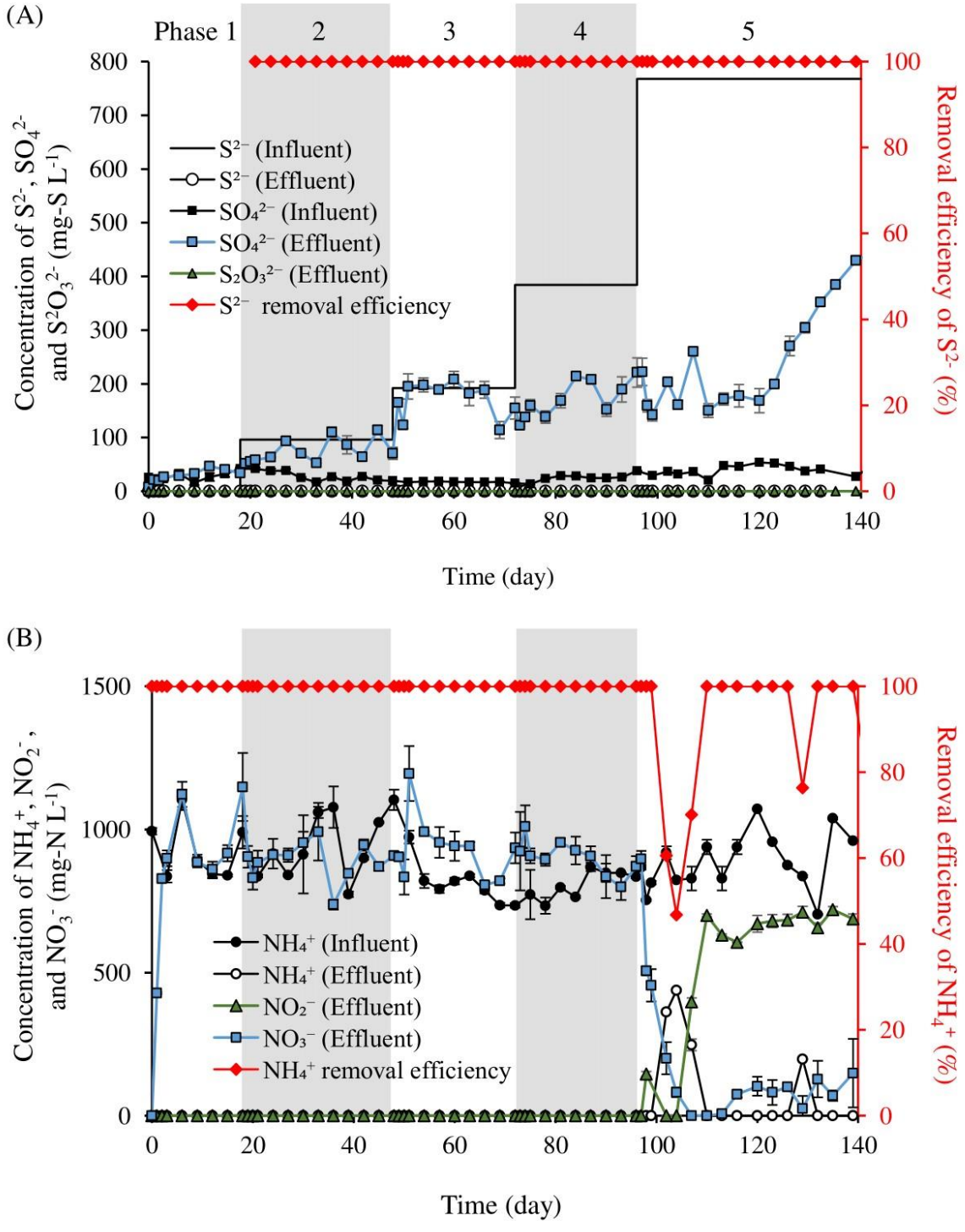


Figure 2-4. Time course of sulfur (A) and nitrogen (B) compounds concentration of influent and effluent.

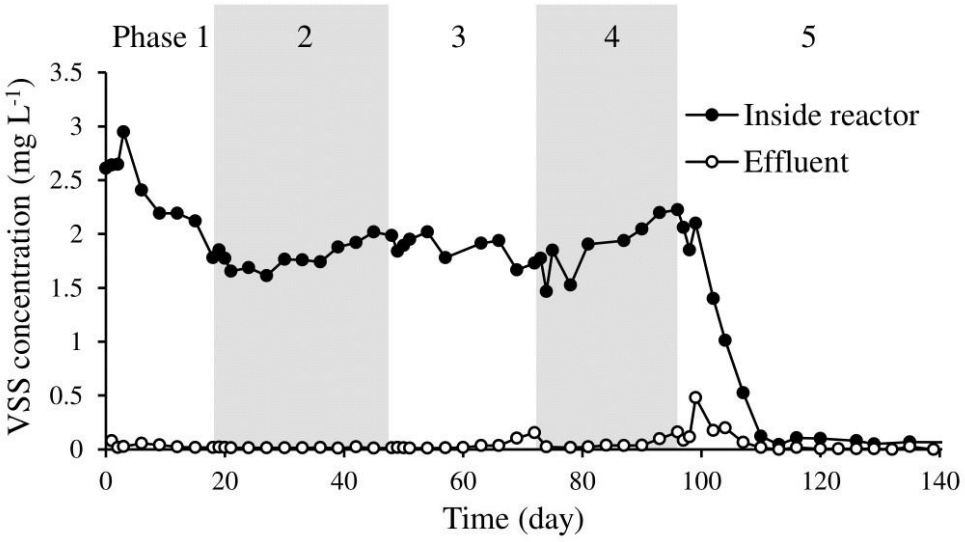


Figure 2-5. Time course of volatile suspended solids (VSS) concentration inside reactor and effluent.

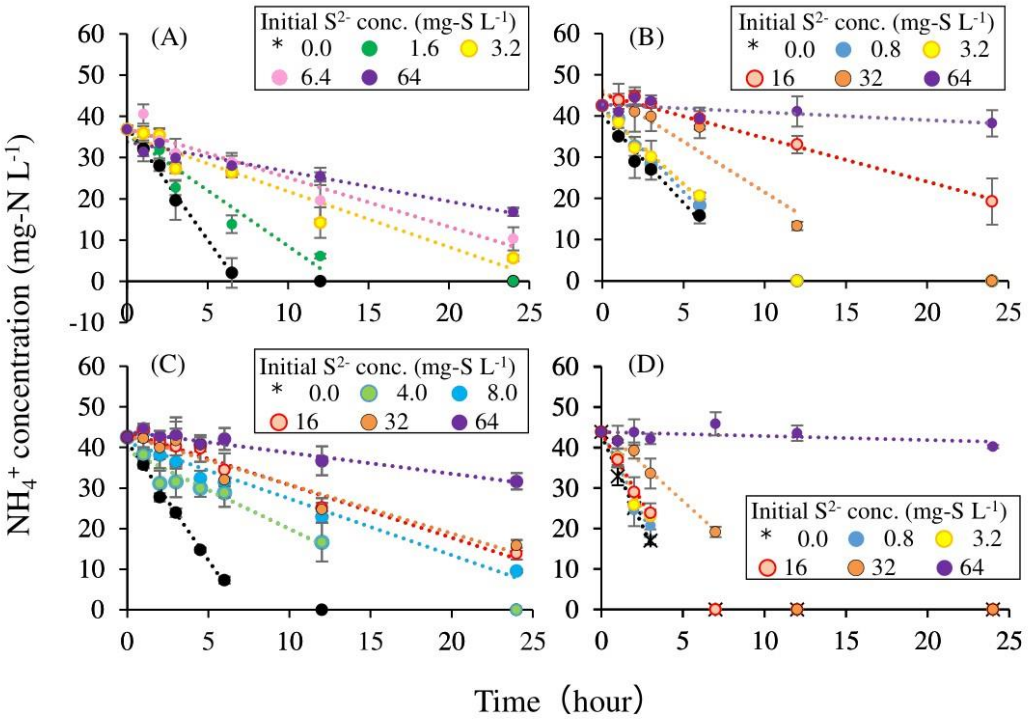


Figure 2-6. Time course of ammonium concentration in batch bioassays using sludge taken from the reactor at the end of Phase 1 (A), Phase 2 (B), Phase 3 (C) and Phase 4 (D). Dotted lines represent the results of the linear regression of experimental data above the detection limit. All regressions are statistically significant at $p<0.01$ except for Phase 3 and 4 under 64 mg-S L^{-1} of initial S^{2-} concentration.

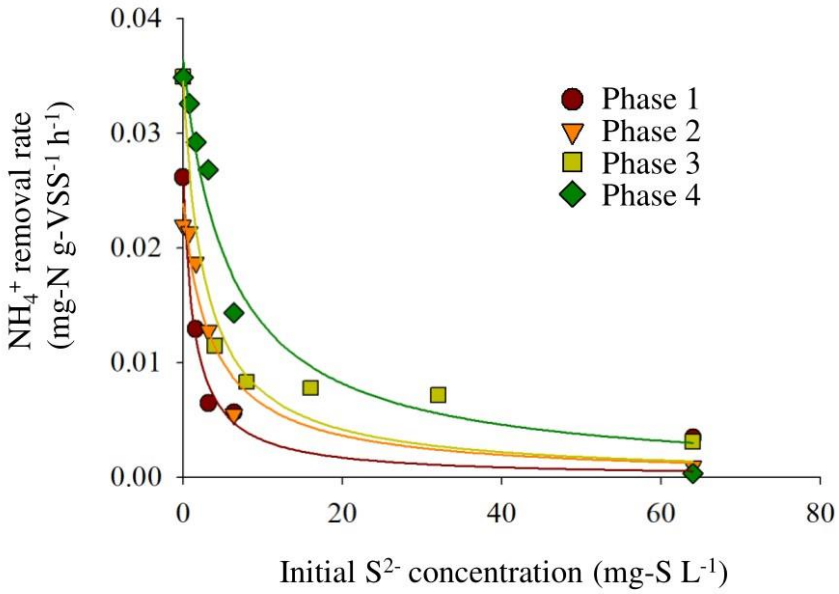


Figure 2-7. Relationships between initial S^{2-} concentrations and NH_4^+ removal rates of the sludge taken from the reactor at the end of each phase. Solid lines represent the significant results of Eq. (2-2) to the experimental data (p<0.05).

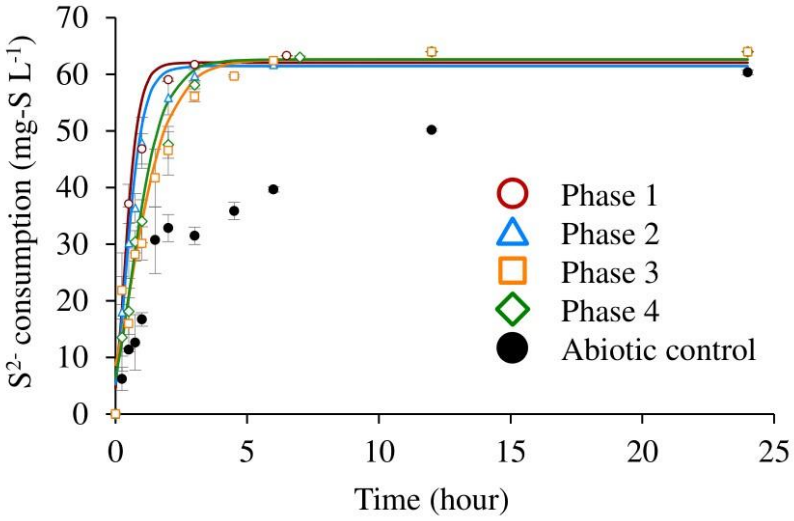


Figure 2-8. S^{2-} consumption rate of the sludge taken from the reactor at the end of each phase. Initial S^{2-} concentration was 64 mg-S L^{-1}. Solid lines represent the significant fitting results of Eqs. (2-3) and (2-4) to the experimental data (p<0.001).

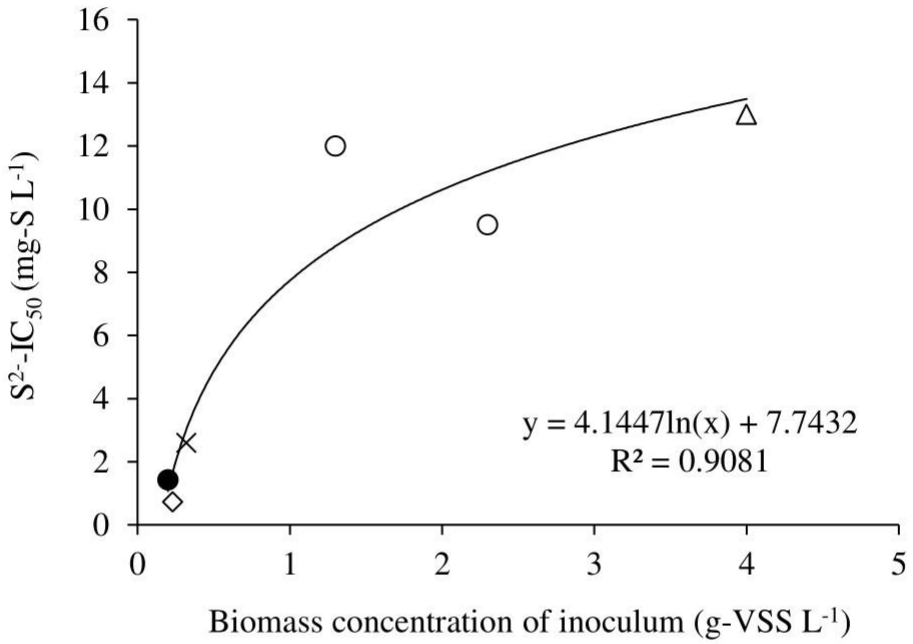


Figure 2-9. Relationship between biomass concentration and 50% inhibitory S^{2-} concentration (S^{2-}-IC_{50}) for NH_4^+ oxidization rate of nitrifying sludge. ○: Delgado Vela et al. (2018); ×: Bejarano-Ortiz et al. (2012); △: Beristain-Cardoso et al. (2010); ◇: Sekine et al. (2018); ●: the present study (0 mg-S L^{-1} d^{-1}, Phase 1).

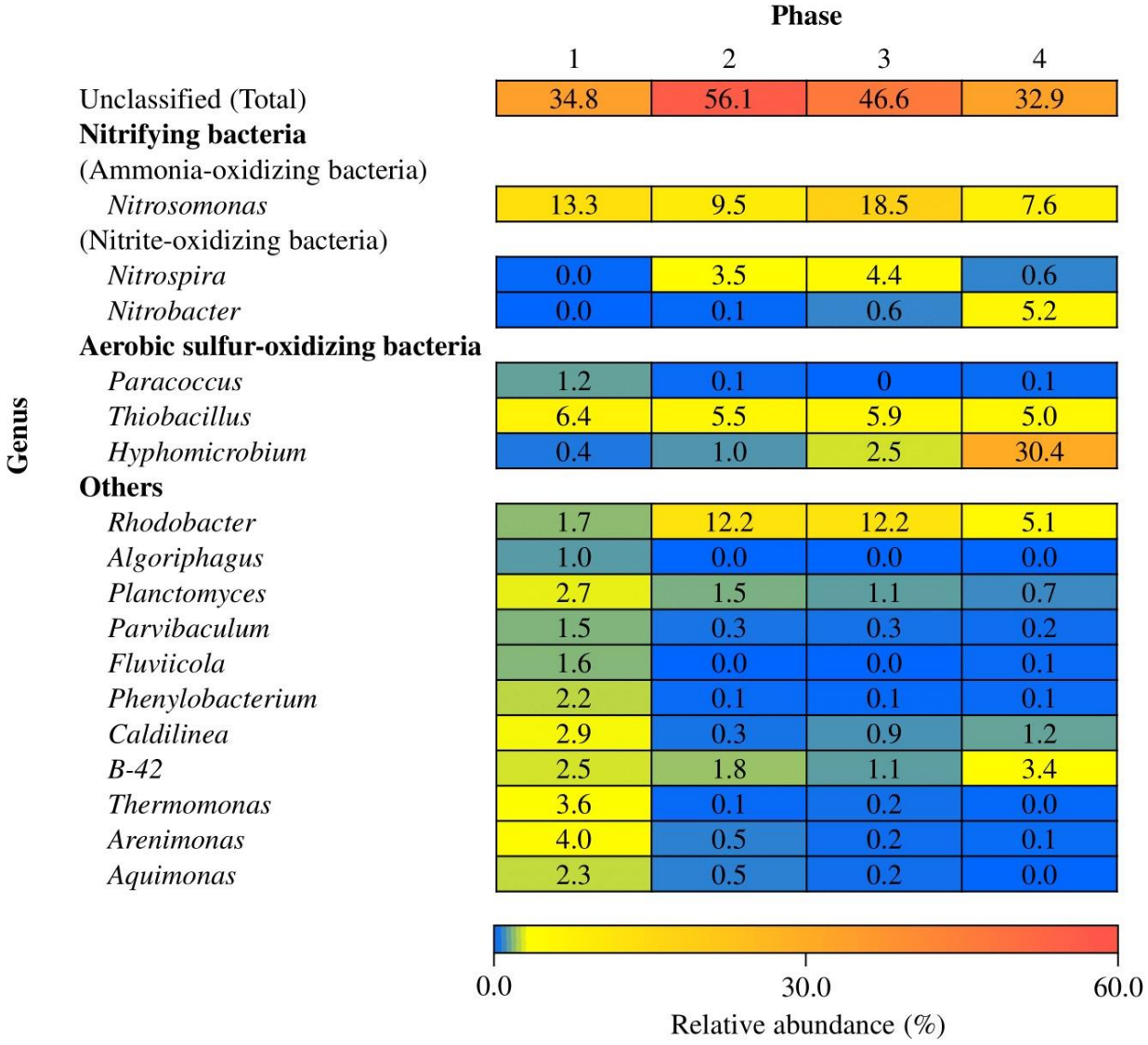


Figure 2-10. Heat map of the relative abundance of the genera which was present more than 1% in the sludge taken from the reactor at the end of each phase at least once. The indicator on the lower denotes the relationship between the relative abundance of each genus and color range.

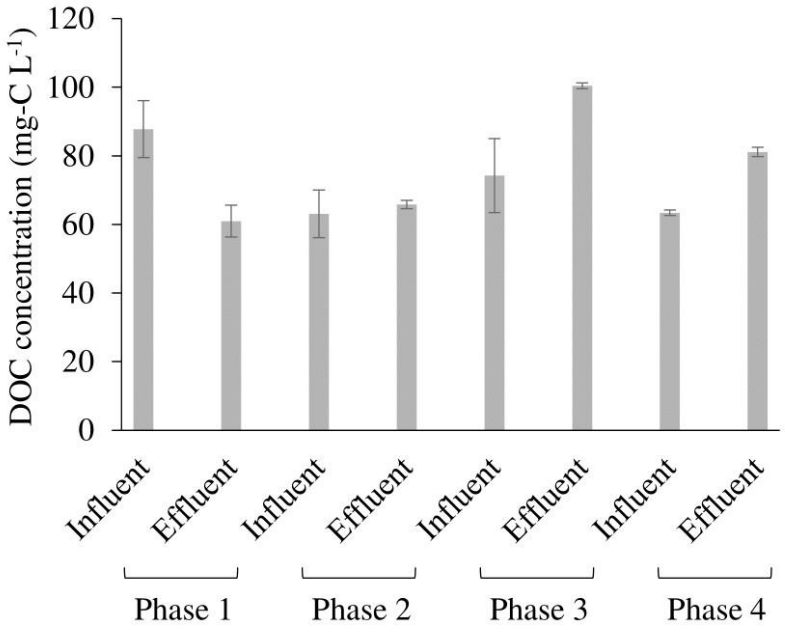


Figure 2-11. Time course of dissolved organic carbon (DOC) concentration of influent and effluent at the end of each phase.

Chapter 3

Integration of biogas desulfurization and ADE nitrification

3.1. Introduction

The composition of biogas produced from anaerobic digestion (AD) depends on the substrate composition and AD treatment process, but, is generally consisted of 40–75% CH_4, 25–55% CO_2, and small quantities of other gases such as N_2, O_2, H_2O, H_2S, and NH_3 (Kadam and Panwar, 2017). Since biogas contains a high concentration of CH_4, it is used as renewable energy (Angelidaki et al., 2018). Nowadays, the size of the biogas market is growing. The global capacity for power generation from biogas facilities is expected to be more than double in a decade, from 14.5 GW in 2012 to 29.5 GW in 2022 (Pike Research, 2012). Produced biogas in 2018 was used around 60% to generate electricity and heat, 30% for cooling and heating in buildings, and less than 20% to inject into the natural gas grid as biomethane (IEA, 2020). Before this biogas utilization, biogas purification is necessary until the required purification level according to each application (Table 3-1; Sun et al., 2015; Kadam and Panwar, 2017; Ullah Khan et al., 2017). Here, three challenges to achieving the simultaneous desulfurization–nitrification (SDN) treatment of biogas were mentioned: 1) desulfurization efficiency, 2) contamination of other gases into biogas during the purification process, and 3) effect of components of biogas on SDN microbes.

Biogas purification consists of cleaning (removal of harmful and toxic compounds) and upgrading (an increase of CH_4 concentration by CO_2 removal). Especially, the cleaning process is necessary in all cases of biogas utilization. Desulfurization is classified as a cleaning process. Regarding the toxicity of H_2S, there are severe health risks for human, i.e., nausea, tearing of the eyes, headaches or loss of sleep by long time exposure at H_2S concentration of more than 2–5 ppm, loss of consciousness, and possibly death in 30 minutes to1 hour at 500–700 ppm, and cessation of respiration and death within a few minutes at more than 700 ppm (Malone Rubright et al., 2017). The upper limit of H_2S concentration for the utilization is 10 ppm for the domestic stove, 250 ppm for on-

site biogas use in boilers for heat generation, 200–1800 ppm for use in internal combustion engines for combined heat and power generation (CHP), and 1–10 ppm to inject CH_4 into natural gas grids and as a vehicle fuel (Muñoz et al., 2015; Sun et al., 2015).

In both the cleaning and upgrading process of biogas, not only the removal efficiency of contaminants from biogas but also the suppression of mixing of other gases from the outside should be considered well for biogas utilization. The dilution of biogas causes a reduction of the heating value. And also, in particular, O_2 contamination increases the inflammability of biogas and causes equipment corrosion. Limiting O_2 concentration (LOC) of CH_4 is about 13% under room temperature and atmospheric pressure, thus if the concentration of O_2 is more than 13%, a mixed gas of CH_4 and O_2 explodes depending on the concentration of CH_4 (Ota, 2014). The stringent quality requirements are encountered in biomethane for injection into natural gas grids and as a vehicle, which often demands less than 0.2–0.5% of O_2 (Muñoz et al., 2015). O_2 supply into the reactor is necessary for the SDN process, but O_2 (or air) easily contaminates into biogas in the SBR used in chapter 2. Therefore, in chapter 3, biogas treatment using the continuous stirred tank membrane reactor (CSTMR) with an external O_2 bubbling column (Figure 3-1) was attempted to desulfurize biogas with high treatment efficiency without less contamination of other gases, especially O_2. In the SBR operation, the liquid level fluctuates in an operation cycle due to the separation of filling and discharge periods, e.g. liquid level of SBR used in Chapter 2 was one third less at the beginning of the reaction period compared with it at the end of the period, leading the fluctuation of the biogas contacting time with liquid and caused the unstable quality of exhausted gas when biogas is supplied from the bottom of the reactor. By contrast, in the CSTMR operation, the liquid was maintained at a certain level throughout the whole period since the effluent was continuously discharged with the influent supply, and high sludge retention time and gradual substrate supply which are found to be needed for a stable SDN process in chapter 2 can be applied. Effluent discharged through a membrane module may be directly used for microalgal cultivation without bacterial contamination. Furthermore, the operation of the reactor in a closed system and O_2 supply through the external O_2 bubbling column contributes

to limit O_2 contamination into desulfurized gas. When 100% O_2 is supplied instead of air, N_2 contamination from the air should also be avoided.

Lastly, for the treatment of biogas in the SDN process, other than H_2S, the effect of different compounds in biogas on the biological desulfurization and nitrification should be investigated to maintain high treatment efficiency. CO_2 accounting for around 40% of biogas is a required compound for the growth of autotrophic organisms including a part of nitrifying bacteria and sulfur-oxidizing bacteria to assimilate carbon through the Calvin-Benson cycle. In contrast, it has been well known that a high concentration of CO_2 is an inhibitor of the microorganism in general. The advocated main inhibition mechanisms were as follows (Dixon and Kell, 1989): (1) Interaction of CO_2 with lipids of the cell membrane, which changes the function of the cell membrane and leads to disturbing to the uptake of various nutrients and growth; (2) the pH reduction in the environment; (3) Break of intracellular ion balance by the penetration to the inside of the cell and dissociation; (4) Feedback inhibition on decarboxylation reactions. Therefore, there is a possibility that nitrifying bacteria and sulfur-oxidizing bacteria will be inhibited by supplying biogas containing CO_2. However, these inhibition strength depends on the microorganism species and cultivation condition. In the previous study related to the storage method of foods, *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia entercolitica* were inoculated to beef meat and cultivated under different temperatures after vacuum packaging or 100% CO_2 purge (Gill and Reichel, 1989). Then, the growth of all bacteria was completely stopped under 100% CO_2 purge and at 2^{o}C conditions. Besides, there was no difference in *Listeria monocytogenes* growth between vacuum packaging and 100% CO_2 purge at 10^{o}C. Another species also maintained a growth rate of approximately 60%. However, regarding the effect of CO_2 exposure on nitrification activity, most of the previous studies focused on the enhancement of the activity by CO_2 (or dissolved inorganic carbon, DIC) supply (Jun et al., 2000; Green et al., 2002; Wett and Rauch, 2003; Guisasola et al., 2007) because nitrifying bacteria are chemolithoautotrophs, which assimilate CO_2 via the Calvin-Benson cycle to meet the carbon requirement for growth. Peng et al. (2015) reported that four times higher NH_4^+ oxidation rate was obtained under 200 mg-C L^{-1} of DIC

concentration compared with 8-mg-C L^{-1} DIC concentration condition. To treat biogas in the SDN process, it is necessary to reveal the effect of higher CO_2 concentrations that simulate a biogas composition on nitrification activity.

In this chapter, to establish simultaneous biogas and anaerobic digestion effluent (ADE) treatment by the SDN process, the CO_2 inhibition effect on nitrification was investigated in a batch bioassay. Then, the SDN treatment efficiency and also O_2 contamination level into biogas were evaluated in the continuous synthetic biogas and ADE treatment using CSTMR with the external O_2 bubbling column.

3.2. Materials and Methods

3.2.1. ADE used as substrate and inoculum

The same ADE and nitrifying sludge as those in section 2.2.1, chapter 2 were used as a substrate and inoculum, respectively. The pre-treatment methods of each sample were also the same as those in section 2.2.1, chapter 2.

3.2.2. Evaluation of CO_2 inhibitory effect on nitrification in batch bioassay

To evaluate the CO_2 tolerance of nitrification, a bioassay was conducted after CO_2 gas supply with different concentrations. 0.45-μm filtered ADE was used after 12 times dilution and 11.9-g-C L^{-1} 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) addition as a pH buffer; as a result, NH_4^+ concentration was approximately 75 mg-N L^{-1}. pH was adjusted to 8.1 by HCl addition. A 160-mL serum bottle was used as a container. The nitrifying sludge was added into the bottle after bubbling gas with different CO_2 concentration (5, 10, and 20% CO_2, 60% O_2, and N_2 (base)) in the substrate for 10 minutes, and then, the bottle was immediately closed with rubber cap and aluminum seal. The total is 100 mL of effective volume (94.5 mL of the above ADE substrate, plus 5 mL of the sludge). Initial volatile suspended solids (VSS) concentration was adjusted to 0.22 g- VSS L^{-1}. Abiotic control which was added ultrapure water instead of the sludge was also prepared. The assay was conducted for 72 hours under 30±1°C temperature and 160 rpm agitation using a thermostat shaker.

All assays were performed in triplicate.

Before and after the CO_2 gas bubling, DIC concentration of medium was analyzed by the catalytic oxidation method (TOC-L_{CPN}; Shimadzu, Japan). During the assays, the NO_2^-, and NO_3^- concentrations in all conditions were periodically measured using HPLC with conductometric detectors (CDD-$10A_{VP}$; Shimadzu, Japan) and two types of the column: IC YS-50 for cation analysis and IC NI-424 for anion (Shodex series; Showa Denko, Japan). After the experiment, the dissolved oxygen (DO) concentration and pH were measured using a DO probe (InLab 605; Mettler Toledo, USA) and a desktop pH meter (SevenCompact pH/Ion meter S220; Mettler Toledo, USA), respectively. NO_2^- and NO_3^- removal rates were obtained by linear regression of the time-course change of these concentrations.

3.2.3. Simultaneous biogas desulfurization and ADE nitrification

ADE and synthetic biogas without CO_2 (0, 0.125, 0.25, and 0.5 % H_2S, N_2 base instead of CH_4) were treated by an CSTMR containing nitrifying sludge, consisted of 3.0-L CSTR, an external membrane cartridge filter (0.22 μm pore size; UPM-053; Asahi Kasei, Japan) for discharge, and 50-mL O_2 bubbling column (Figure 3-1). pH, temperature and HRT were controlled to the same condition by the same method as SBR operation in chapter 2. To change the H_2S concentration of synthetic biogas during the experimental period, two gas cylinders with flow regulators were prepared for pure N_2 gas and 0.5% H_2S gas (N_2 base). These gases were continuously supplied together into the reactor through a bifurcation tube (I.D. 2.5 mm) with a total of 32 mL min^{-1} of flow rate. Thus, NH_4^+ loading rate (NLR) was approximately 260 mg-N L^{-1} d^{-1} and S^{2-} loading rate (SLR) was stepwise increased from 0 to 25, 50, 100 mg-S L^{-1} d^{-1} (Phase 1–4, Figure 3-2) in the range of values in the actual biogas and ADE treatment assumed in section 2.3.4., chapter 2. DO was adjusted to 3.0 mg-O_2 L^{-1} by O_2 supply from the gas bag that stores pure O_2 into the O_2 bubbling column using an air pump. Undissolved O_2 was restored in the same gas bag. On-off of the air pump was controlled by USB relay module driving using a program written in the Python language and DO value inside the reactor recorded in desktop PC through an optical DO meter (Seven2Go DO meter S9; Metler Toledo, USA).

As analytical parameters, H_2S, O_2, and CO_2 concentrations in the exhausted gas, the NH_4^+, NO_2^-, NO_3^-, S^{2-}, $S_2O_3^-$, SO_4^{2-}, DIC concentrations of the influent and effluent, and suspended solids (SS) and VSS concentrations of the reactor inside were measured once per 3 days. The NH_4^+, NO_2^-, NO_3^-, S^{2-}, $S_2O_3^{2-}$, SO_4^{2-} and DIC concentration were analyzed using the same HPLC and TOC analyzer as section 3.2.2. O_2 and CO_2 concentrations were measured by gas chromatography with a TCD detector and a column (Shincarbon-ST 50/80; Shinwa chemical, Japan). Other parameters were measured using the same method as section 2.2.3. in chapter 2.

A batch bioassay and bacterial community analysis were conducted using the reactor sludge at the end of each SLR phase with almost the same method of section 2.2.4 in chapter 2. Note that the reactor sludge was used after centrifugation at 3000×g for 10 min and the replacement of the supernatant with distilled water to remove any dissolved compounds in the batch bioassay. In addition to NH_4^+ and S^{2-}, NO_2^- and NO_3^- concentrations were periodically measured. In the bacterial community analysis of the sludge samples, the 16S rRNA gene V4 region of the extracted DNA sample was amplified through the MiSeq (Illumina, San Diego) sequencing platform of Bioengineering Lab Co., Ltd. (Kanagawa, Japan) after the DNA extraction. In the Quantitative Insights Into Microbial Ecology 2 software package (2020.8 release), all the effective sequences were grouped into the operational taxonomic units (OTUs). Each OTU was classified using a 97% OTU reference 16S rRNA database of Greengenes 13_8 (greengenes.lbl. gov).

3.3. Results and discussion

3.3.1. Evaluation of CO_2 inhibitory effect on nitrification in batch bioassay

After the bubbling of gas containing CO_2 at 5, 20, and 40%, liquid pH was maintained at 7.02–7.91 since HEPES buffer was added. The DIC concentration of the substrate consisted of 12-times diluted ADE was changed from 45.5 mg-C L^{-1} to 50±0.11, 219±1.7, and 371±2.9 mg-C L^{-1}, respectively, in each CO_2 concentration condition. At the end of the experiment, DO was maintained at more than 9.31 mg-O_2 L^{-1}. pH was between 6.93 and 7.59 in every condition. During the experiment,

the NO_3^- concentration increased linearly with time (p<0.01 in all conditions, Figure 3-3). NO_3^- production rate calculated from the time-course change of NO_3^- concentration was 5.12, 3.29, and 1.97 mg-N g-VSS^{-1} h^{-1}. In a condition supplied 40% CO_2 gas which is almost the same concentration as biogas, NO_3^- production rate decreased by 62% compared with that in 5% CO_2 condition. A similar inhibition effect of CO_2 was reported in previous study (Denecke and Liebig, 2003). They revealed that the NOx-N production rate decreases by 18% under 9.7 % CO_2 supply (340 mg-DIC L^{-1} in liquid phase) compared with when 1.5 % CO_2 was used. Based on these results, supplying a high concentration of CO_2 gas (approximately more than 200 mg-DIC L^{-1}) is not appropriate to maintain the high treatment efficiency of the SDN process. Although the limitation of DIC also suppresses the nitrification activity, a certain amount of DIC will, fortunately, be supplied from ADE. Therefore, in the SDN process, CO_2 should be removed from biogas in advance, and it is recommended to monitor the DIC concentration of the reactor to avoid a lack of DIC.

3.3.2 Treatment efficiencies and O_2 contamination into biogas in the SDN of biogas and ADE

In the SDN treatment of synthetic biogas consisted of N_2 and H_2S, and ADE using CSTMR, both desulfurization and nitrification efficiencies were maintained at 100% under 0–100-mg-S L^{-1} d^{-1} of SLR and 263-mg-N L^{-1} d^{-1} of NLR. S^{2-} and $S_2O_3^{2-}$ in effluent and H_2S in exhausted gas were not detected (Figure 3-4, (A)). SO_4^{2-} concentration of effluent increased with SLR. The amount of produced SO_4^{2-} was almost the same as the amount of supplied H_2S into the reactor at 0–5000 ppm, that is, all supplied H_2S was totally dissolved into a liquid phase and oxidized to SO_4^{2-} in the CSTMR in contrast to SDN treatment using the SBR in chapter 2 (section 2.3.1) (Figure 3-5). S^0 accumulation requiring high-maintenance is a major issue in the bio-desulfurization. This S^0 production is generally caused by less the number of bacteria and/or oxygen supply per SLR in addition to characteristics of bacteria such as *Hyphomicrobium* detected in SBR in chapter 2. However, sufficient number of bacteria and O_2 supply can be easily maintained in the SDN treatment because SLR is reduced from 950–4400 mg-S L^{-1} d^{-1} of conventional bio-desulfurization (Muñoz et al., 2015) to less than 100 mg-S L^{-1} d^{-1} by combining biogas desulfurization with ADE nitrification. Additionally, as discussed

below (section 3.3.4), difference of dominant sulfur-oxidizing bacterial species was confirmed between SBR in chapter 2 and CSTMR. This complete H_2S oxidation without accumulation of S^0 could be considered as an advantage of SDN treatment using CSTMR. ADE was also oxidized to NO_3^- completely without NH_4^+ and NO_2^- accumulation by S^{2-} inhibition (Figure 3-4, (B)). Therefore, it was demonstrated that stable biogas desulfurization and ADE nitrification can be achieved simultaneously using the CSTMR.

As mentioned previously, contamination of O_2 into biogas can be a reason of corrosion of equipment in post-processes such as compressors, pipelines, and gas storage tank, and is entail explosion hazards at more than 13% of O_2 concentration. To utilize biogas as an alternative to natural gas or a vehicle fuel, O_2 concentration of less than 0.2–0.5% is often required (Muñoz et al., 2015). In this experiment, the O_2 concentration of exhaust gas (desulfurized synthetic biogas) fluctuated from 0.14% to 0.64% throughout the operation period; the average is 0.38±0.11% (Figure 3-6). Because O_2 gas was supplied into the reactor through an external O_2 bubbling column, this contaminated O_2 must have been derived from the dissolved O_2 in the liquid phase. In this reactor, DO was adjusted to 3.0 mg-O_2 L^{-1} during the reactor operation because it was mentioned that the optimum DO concentration of NO_2^- oxidizing bacteria is higher than 2.0 mg-O_2 L^{-1} (Ruiz et al., 2007; Blackburne at al., 2008). Therefore, although O_2 contamination may be suppressed by decreasing DO concentration inside the reactor, 3.0 mg L^{-1} or at least 2.0 mg L^{-1} is appropriate for achieving high treatment efficiency of biogas and ADE and further utilizing desulfurized biogas.

3.3.3. S^{2-} tolerance and removal rate of sludge in each S^{2-} loading rate phase

The SS and VSS concentrations of the sludge inside the reactor (Figure 3-7) decreased from 4.09 g L^{-1} and 3.29 g L^{-1} to 2.58 g L^{-1} and 1.87 g L^{-1}, respectively, during the first 9 days. Then, these concentrations were maintained at 3.01±0.53 g L^{-1} and 2.05±0.49 g L^{-1}, respectively. Using a part of the sludge inside the reactor, batch bioassays were conducted at the end of each SLR phase, and S^{2-}-IC_{50} for nitrification was evaluated. After 24 h batch bioassays, the DO concentration was more than 11 mg-O_2 L^{-1}, and pH was between 6.85 and 7.85 in every condition. During the experiment, the

NH_4^+ concentration decreased, and NO_3^- concentration increased linearly with time. NH_4^+ removal and NO_3^- production rates of sludge in all SLR phases tended to decrease with an increase in initial S^{2-} concentration. With an increase in the SLR phase, both reactions began to occur even under the condition of higher initial S^{2-} concentration. NO_2^- tended to accumulate in the condition with high initial S^{2-} concentration. Estimated S^{2-}-IC_{50} (p<0.05) increased with phases from 0.84 mg-S L^{-1} to 2.35, 4.00, and 15.79 mg-S L^{-1} for NH_4^+ removal efficiency ($\fallingdotseq NO_2^-$ production efficiency) and 0.32 mg-S L^{-1} to 0.66, 0.70, 2.52 mg-S L^{-1} for NO_3^- production efficiency, respectively (Figure 3-8–3-9, Table 3-2). Surprisingly, these increases in S^{2-}-IC_{50} for NH_4^+ removal and NO_3^- production efficiencies were 19 times and 8 times, respectively, which were higher than a 4-time increase in S^{2-}-IC_{50} for NH_4^+ removal efficiency in chapter 2 using SBR with a long fill period. However, note that both NH_4^+ removal and NO_3^- production rates of sludge under 0 mg-S L^{-1} of S^{2-} initial concentration suddenly decreased in Phase 4. In this batch experiment, the S^{2-} concentration was completely removed within 1 hour in all bioassays with 64 mg-S L^{-1} of initial S^{2-} concentration (Figure 3-10). This reduction of S^{2-} was faster than the experiment using SBR sludge in chapter 2 at where removal of 64-mg-S L^{-1} took 3–6 hours. Accordingly, the time-course change of S^{2-} was not fitted with the integrated Gompertz model (equation (2-3)–(2-4) in section 2.2.4.) which is for estimation of the maximum S^{2-} removal rate; however, the change showed faster S^{2-} removal in the condition using sludge in latter phases. Therefore, this increase in S^{2-} removal rate must have increased the S^{2-} IC_{50} for nitrification ability of sludge in the CSTMR. As a reason for these large increase of S^{2-}-IC_{50} for nitrification, decrease in nitrification rate, and increase in S^{2-} removal rate of sludge, which are different trends from the experiment using SBR in chapter 2 (section 2.3.2), it might be considered that CSTMR characteristics such as higher sludge retention ability and shear/agitation stress to microbes by using membrane module and pump changed microbial community structure.

3.3.4. Bacterial community dynamics

We obtained 33,543–42,754 high-quality reads from each of the four communities for a total of four samples taken at the end of each phase. The observed OTUs were classified to phylum-,

class-, order-, family-, genus-, and species-level with 99.5–99.9%, 92.7–98.0%, 90.8–96.3%, 62.7–84.0%, 13.4–27.6% and 0.34–0.86% of identification rate, respectively. Figure 3-11 shows the result of OUT classification. Unclassified genera were also listed in Figure 3-11 with order or family names because OTUs that were present more than 1% and classified at genus level were only two: *Nitrospira* sp. and *Thiobacillus* sp. A detected unclassified genus belongs to the family Nitrosomonadaceae which has genera *Nitrosomonas* and *Nitrosospira*, typical ammonia-oxidizing bacteria. Since the relative abundance of this genus was high (28.5%) in Phase 1, this genus may have contributed to the NH_4^+ oxidation reaction. However, it gradually decreased to 7.6% in the latter phases. The relative abundance of nitrite oxidizing bacteria *Nitrospira* which was present in the former phases in the SBR experiment, was also present in CSTMR and gradually decreased from 5.7% to 0.0%. This genus probably has low S^{2-} tolerance. This decrease in the abundance of species in the family Nitrosomonadaceae and *Nitrospira* might have been a reason for the decrease in the nitrification rate of sludge in Phase 4. Other detected families such as Chitinophagaceae and Xanthomonadaceae also have some heterotrophic ammonia-oxidizing bacteria or nitrite-oxidizing bacteria (Wang et al., 2020), however, it is uncertain whether detected spices in these families have nitrification reaction system.

Regarding sulfur-oxidizing bacteria, the relative abundance of *Thiobacillus* sp. increased from 0.0 to 25.4 maximum in CSTMR in contrast to SBR in which this genus did not increase. On the other hand, the abundance of *Hyphomicrobium* which increased in SBR and probably caused S^0 production did not increase in CSTMR. Therefore, the increase in *Thiobacillus* instead of *Hyphomicrobium* probably the reason for the increase in the S^{2-} removal rate of sludge with SLR and 100% of H_2S conversion efficiency to SO_4^{2-} in the continuous CSTMR operation. At the end of the experiment using CSTMR, a part of the sludge in the CSTMR was transferred to three 50 mL cylinders. After 20 min which is the same duration as the sedimentation period in a cycle of SBR, the settled sludge volume was at 92.7±1.2%. It is known that *Thiobacillus* is small short rods (Pokorna and Zabranska, 2015). Therefore, there is a possibility that *Thiobacillus* that may have low settle ability and be discharged in the SBR operation grew well in the CSTMR operation and contributed

to H_2S oxidation to SO_4^{2-}. Regarding *Hyphomicrobium*, a reported H_2S affinity (Ks) of *Hyphomicrobium* sp. I55 (50 mg L^{-1}; Zhang et al., 1991) is lower than that of *Thiobacillus thioparus* T5 (0.32 mg L^{-1}; De Zwart et al., 1997), which is unfavorable to grow in a continuous feeding reactor. Also, an increase in DOC concentration which will be a substrate for chemolithoheterotroph *Hyphomicrobium* was not shown in the effluent of CSTMR in contrast to in the SBR (Figure 3-12). These situations may have suppressed the growth of *Hyphomicrobium* in the CSTMR.

Based on these results, it was considered that the higher sludge retention ability of CSTMR promoted the increase of *Thiobacillus* abundance with the increase in SLR. And the growth of this genus probably increased the S^{2-} removal rate and the S^{2-} tolerance of nitrification activity of SDN sludge in the CSTMR, more than in the SBR. These increases contributed to the stable and high efficiency of simultaneous biogas and ADE treatment by the SDN process. In the social implementation of the SDN treatment, inoculation of pre-cultivated *Thiobacillus* spp. may reduce a period to acclimatize the reactor to high SLR.

3.3.5. The possible approach of CO_2 removal from biogas using membrane technology

An appropriate removal method of CO_2 from the biogas is necessary to establish the SDN treatment of biogas. The CO_2 removal method has been developed to increase biogas calories to upgrade it as an alternative to natural gas. Some methods are already implemented in full-scale anaerobic digestion systems especially in Europe; water scrubbing, organic physical scrubbing, amine scrubbing, pressure swing absorption (PSA) systems, and membrane technology (Table 3-3). Water scrubbing methods used to be a majority due to the simple operation and low cost, but nowadays, implementation of membrane technology and PSA system which are relatively lower cost and less chemical consumption are increasing out by technology development. CO_2 removal was suppressed by H_2S in the PSA system (Sun et al., 2015). On the other hand, the membrane separates gases only by the deference of molecular size. CO_2 separation system using membrane technology mainly consists of compressions and membrane modules. Because CO_2 molecular size (3.3 Å) is smaller than H_2S (3.6 Å) and CH_4 (3.8 Å) (Shah et al., 2017), membrane technology probably appropriate for the

pre-treatment of SDN.

Some module configurations of CO_2 separation using membrane are shown in Figure 3-13–3-14. Gas separation efficiency depends on the type of membrane and gas pressure derived by the capacity and position of compressors. Based on the experimental evaluation of CO_2 inhibition effect on nitrification rate and previous studies (section 3.3.1.) (Denecke and Liebig, 2003; Peng et al., 2015), low CO_2 gas concentration (less than 200 mg-C L^{-1} of DIC concentration of liquid phase) was expected not to inhibit nitrifying bacteria. Therefore, in the pre-treatment of SDN, more attention should be paid to avoid H_2S and CH_4 contamination to separated CO_2 rather than obtaining high CO_2 removal efficiency. This is because contamination of a non-negligible amount of H_2S into separated CO_2 requires additional desulfurization treatment for separated CO_2, and CH_4 contamination caused a reduction of recovered methane gas amount. Thus, a two-stage membrane module system could be used with the SDN reactor (Figure 3-15). One compressor might enough if we can manage the gas pressure of each membrane module well by using such a gas regulator and check valve. Only the most CO_2 is removed in the first membrane module at lower gas pressure. Then, remained CO_2, H_2S, and CH_4 goes to the SDN reactor, and H_2S is removed at 100%. In the second membrane module, a part of CH_4 is removed with remained CO_2, but removed these gases will be returned to the first module with raw biogas, indicating that CH_4 does not leak. Therefore, it is expected that a high concentration of CH_4 and CO_2 gases are produced separately with 100% H_2S removal using SDN. CH_4 will be an alternative to natural gas. Also, it is possible to use CO_2 for the carbon source of microalgae. However, there is not enough information about the H_2S retaining ability of commercialized CO_2 removal membranes because most previous studies focused on CO_2 removal from desulfurized biogas, and simultaneous removal of CO_2 and H_2S from biogas with post-desulfurization treatment. Chen et al. (2015) reported that generic polyimide has 12 of H_2S/CH_4 and 37 of CO_2/CH_4 selectivity which are relatively lower and higher values, respectively, than other membrane polymers such as cellulose acetate poly(dimethylsiloxane). Therefore, there is a possibility that generic polyimide may have the potential as a membrane for the SDN treatment. Further

characterization and development of membranes are necessary for CO_2 removal from biogas with H_2S and CH_4 retention.

Table 3-1. Major components and concentrations of biogas (Kadam and Panwar, 2017) and standard permissible concentrations for various biogas utilization (Sun et al., 2015; Ullah Khan et al., 2017).

Components	Impact on biogas utilization	Unit	Biogas	Standards for each biogas utilization				
				Boiler	Cooker, domestic stoves	Stationary engine (CHP)	Vehicle fuel	Natural gas grid
Methane (CH_4)	-	vol%	40-75	-	-	-	>96	>70-98
Carbon dioxide (CO_2)	Decreasing calorific value, anti-knock properties of engines, and corrosion	vol%	25-55	-	-	-	<4	<3
Water vapor (H_2O)	Corrosion, damage due to formation of condensate and ice	vol%	0-10	-	-	<3	<3	<1.0-8
Nitrogen (N_2)	Decreasing calorific value, anti-knock properties of engines	vol%	0-5	-	-	-	-	<2-10
Oxygen (O_2)	Corrosion, risk of explosion	vol%	0-2	-	-	-	-	<0.01-3
Hydrogen sulfide (H_2S)	Corrosion, catalytic converter poison, emission and health hazards	ppm	50-5000	<250-1000	<10	<250-1000	<3.3	<1.3-10

Table 3-2. 50 % inhibitory S^{2-} concentration (IC_{50}) for NH_4^+ removal efficiency and NO_3^- production efficiency of the sludge taken from the reactor at the end of each phase.

Phase	S^{2-} -IC_{50} (mg-S L^{-1})	
	for NH_4^+ removal efficiency	for NO_3^- production efficiency
1	0.84	0.32
2	2.35	0.66
3	4.00	0.70
4	15.79	2.52

Table 3-3. Major CO_2 removal methods for biogas, and penetration rate and investment/operation costs of these methods. (Baena-Moreno et al., 2020, modified).

	Water scrubbing	Chemical scrubbing	Physical scrubbing	Pressure swing absorption	Membrane separation
Description	Physical absorption of carbon dioxide and minor contaminants in water at 6-10 bar	Chemical carbon dioxide absorption with a solvent such as monoethanolamide, piperazine, sodium hydroxide or potassium hydroxide	Similar principle than water scrubbing but using organic solvents	Activated carbon molecular sieves retain carbon dioxide, hydrogen sulfide and ammonia	Based on the different size of molecules, carbon dioxide is permeated through a porus membrane while methane is retained over the membrane surface
Penetration in 2018 (%)	35.5	20.6	4.7	16.8	20.6
Approximate investment costs (€/(m^3 h^{-1}) of biomethane)					
Up to 100 m^3 h^{-1}	10100	9500	9500	10500	7500
Up to 200 m^3 h^{-1}	5500	5000	5000	5500	4800
Up to 300 m^3 h^{-1}	3500	3500	3500	3800	3500
Approximate operation costs (ct m^{-3}-biomethane)					
Up to 100 m^3 h^{-1}	14.0	14.4	13.8	12.8	12.5
Up to 200 m^3 h^{-1}	10.3	12.0	10.2	10.1	8.6
Up to 300 m^3 h^{-1}	9.1	11.2	9.0	9.2	7.5

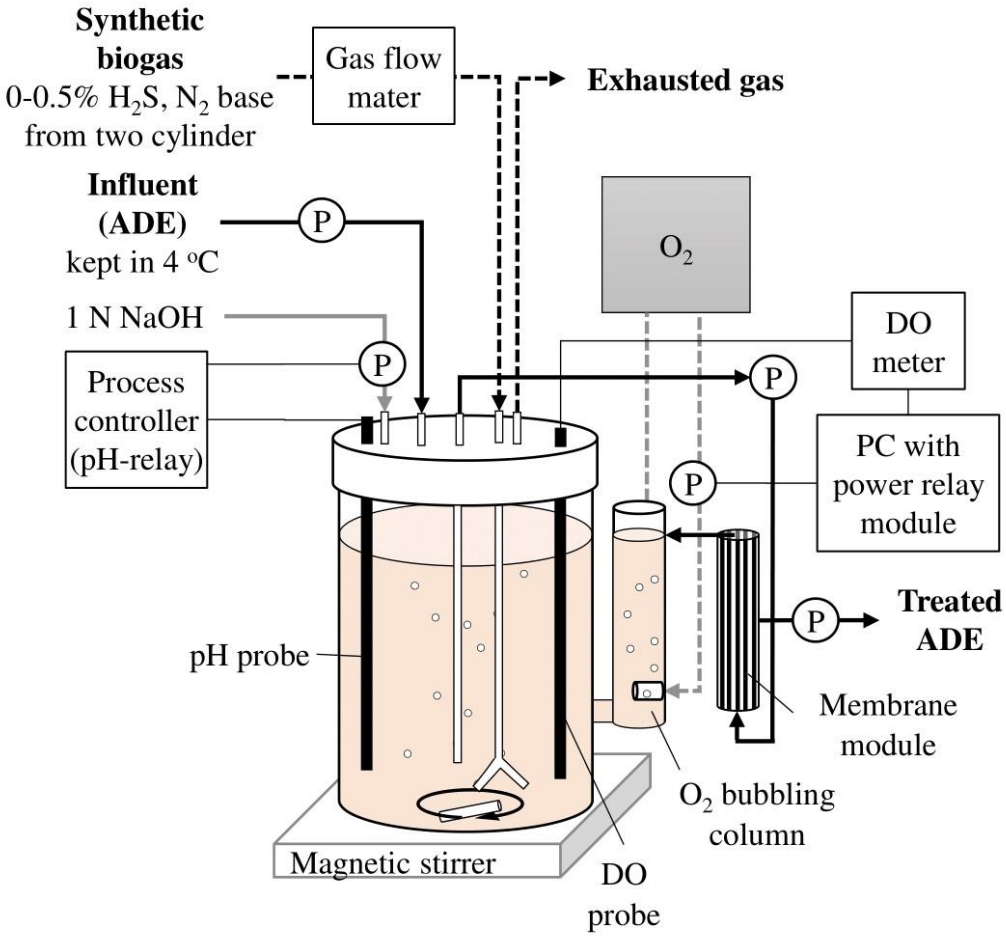


Figure 3-1. Schematic diagram of a continuous stirred tank membrane reactor (CSTMR) with an external O_2 bubbling column.

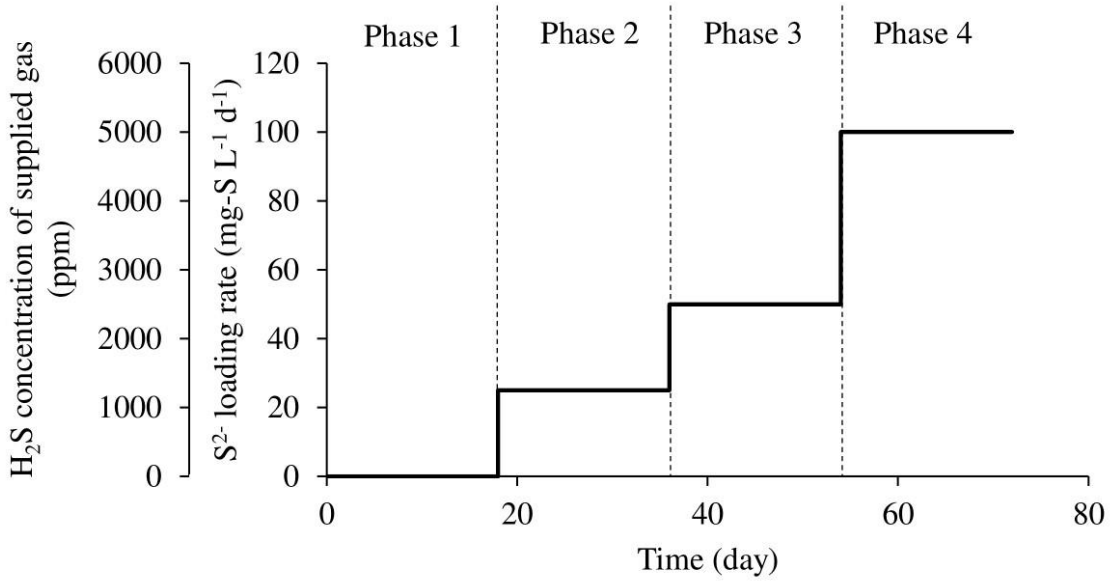


Figure 3-2. Operational schedule of H_2S supply in the reactor.

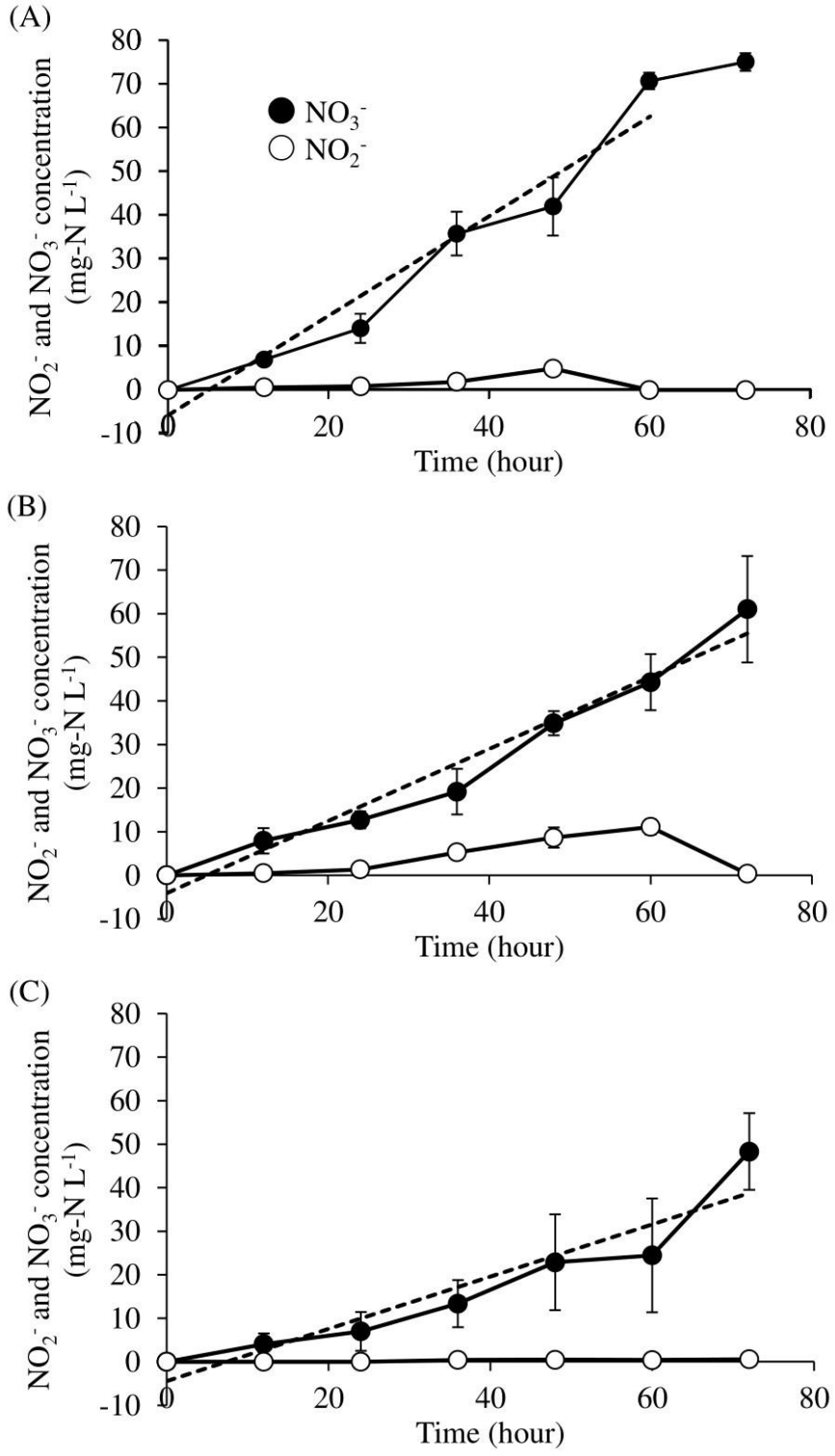


Figure 3-3. Time course of NO_2^- and NO_3^- concentration in each condition supplied different CO_2 concentration gas: 5% (A), 20% (B), and 40% (C). Dotted lines represent the results of the linear regression of experimental data. All regressions are statistically significant at $p<0.01$.

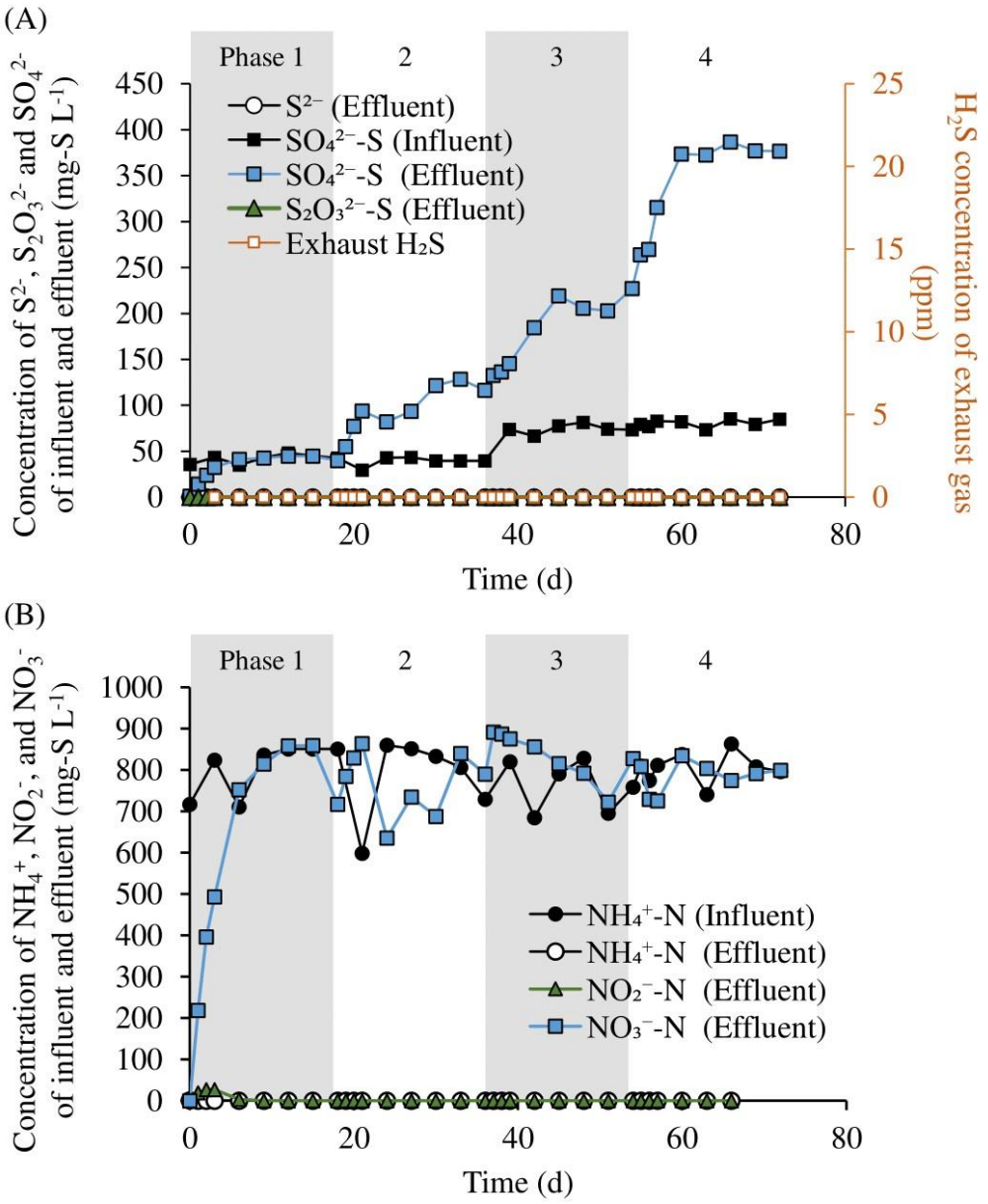


Figure 3-4. Time course of sulfur (A) and nitrogen (B) compounds concentration of influent, effluent and exhaust gas.

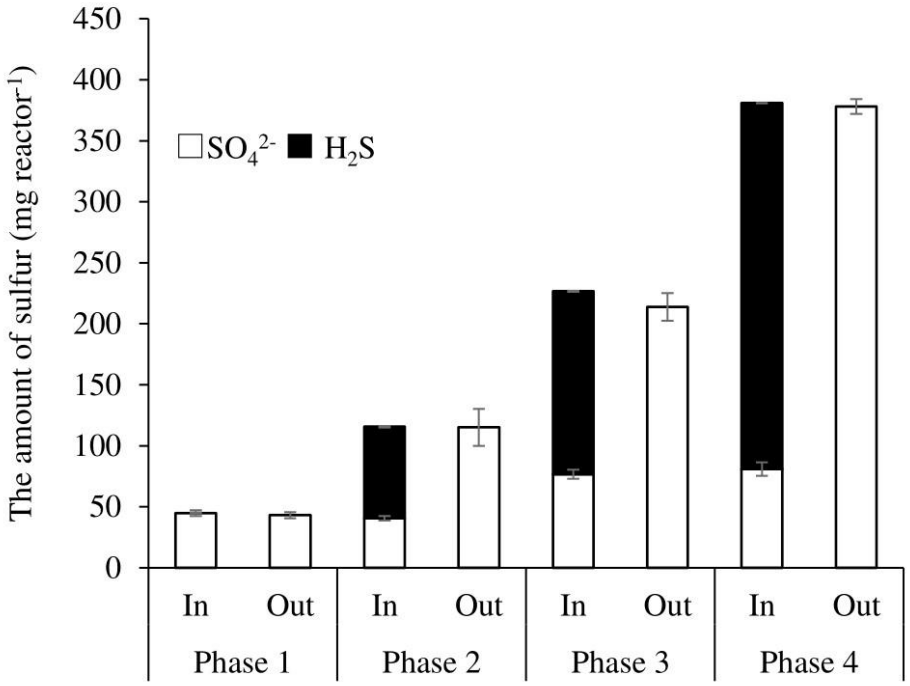


Figure 3-5. Mass-balance of sulfur compounds between input and output in each phase.

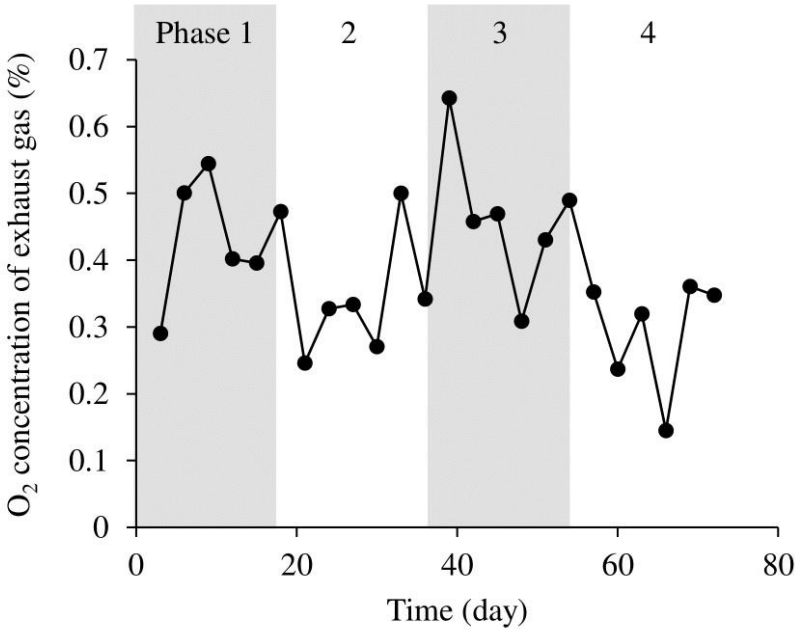


Figure 3-6. Time course of O_2 concentration of exhaust gas.

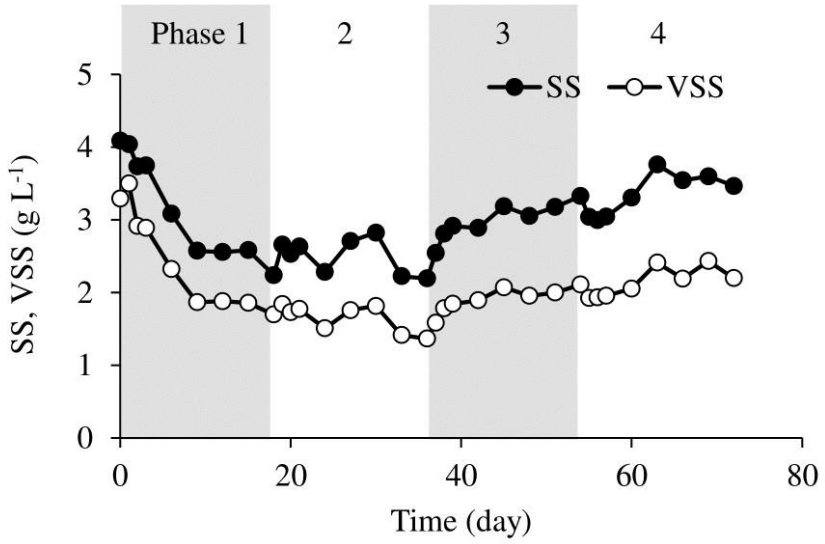


Figure 3-7. Time course of the suspended solids (SS) and the volatile suspended solids (VSS) concentrations inside the reactor.

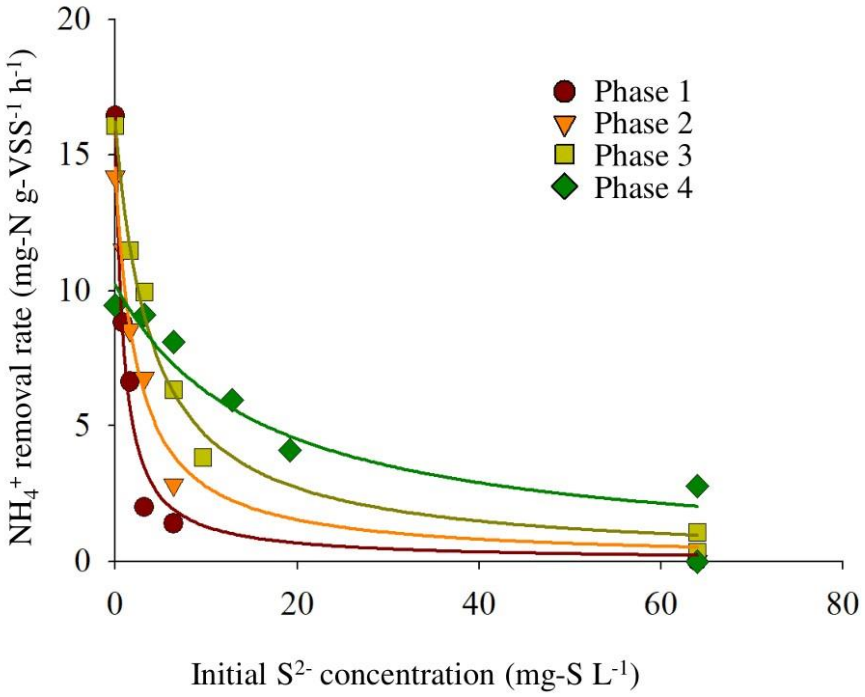


Figure 3-8. Relationships between initial S^{2-} concentrations and NH_4^+ removal rates of the sludge taken from the reactor at the end of each phase. Solid lines represent the significant results of Eq. (2-2) to the experimental data (p<0.05).

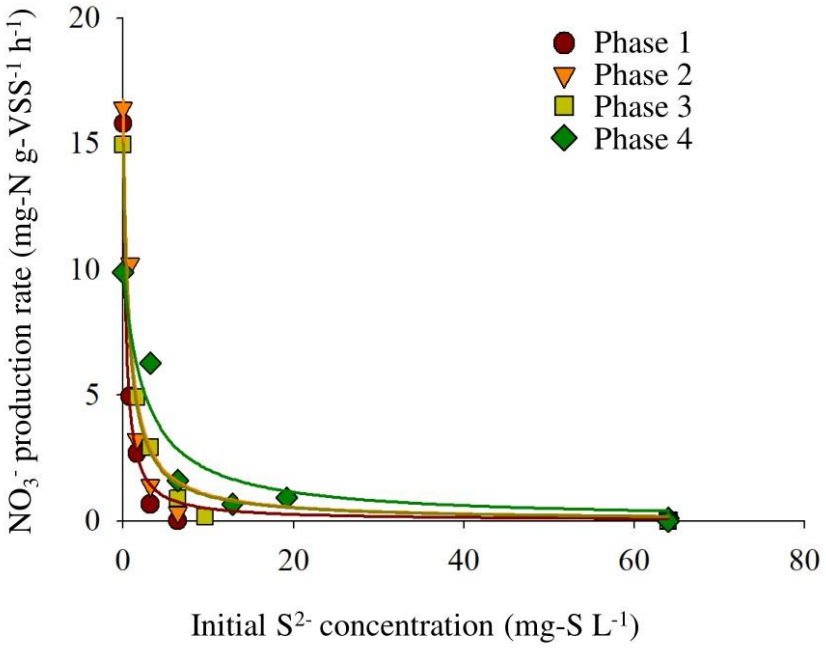


Figure 3-9. Relationships between initial S^{2-} concentrations and NO_3^- production rates of the sludge taken from the reactor at the end of each phase. Solid lines represent the significant results of Eq. (2-2) to the experimental data (p<0.05).

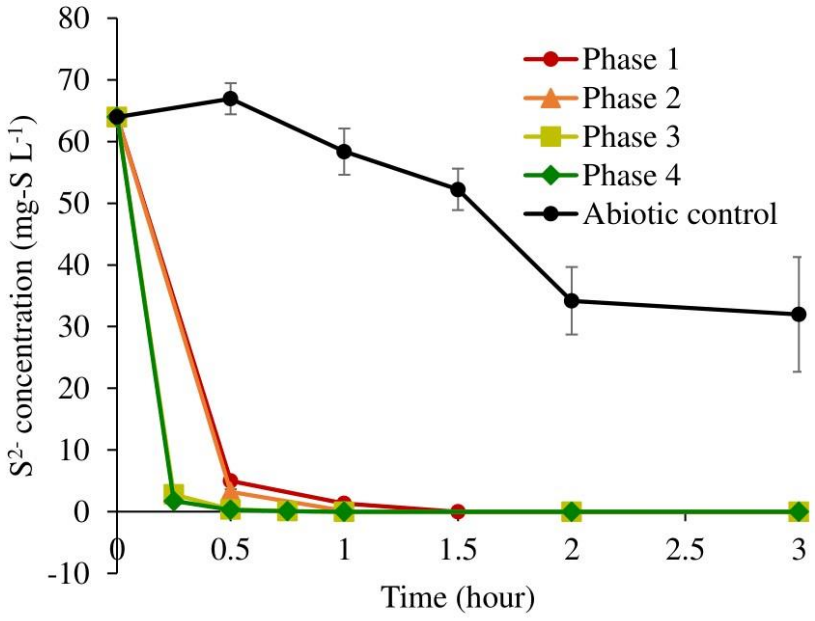


Figure 3-10. Time course of S^{2-} concentration in batch bioassays using sludge taken from the reactor at the end of each phase.

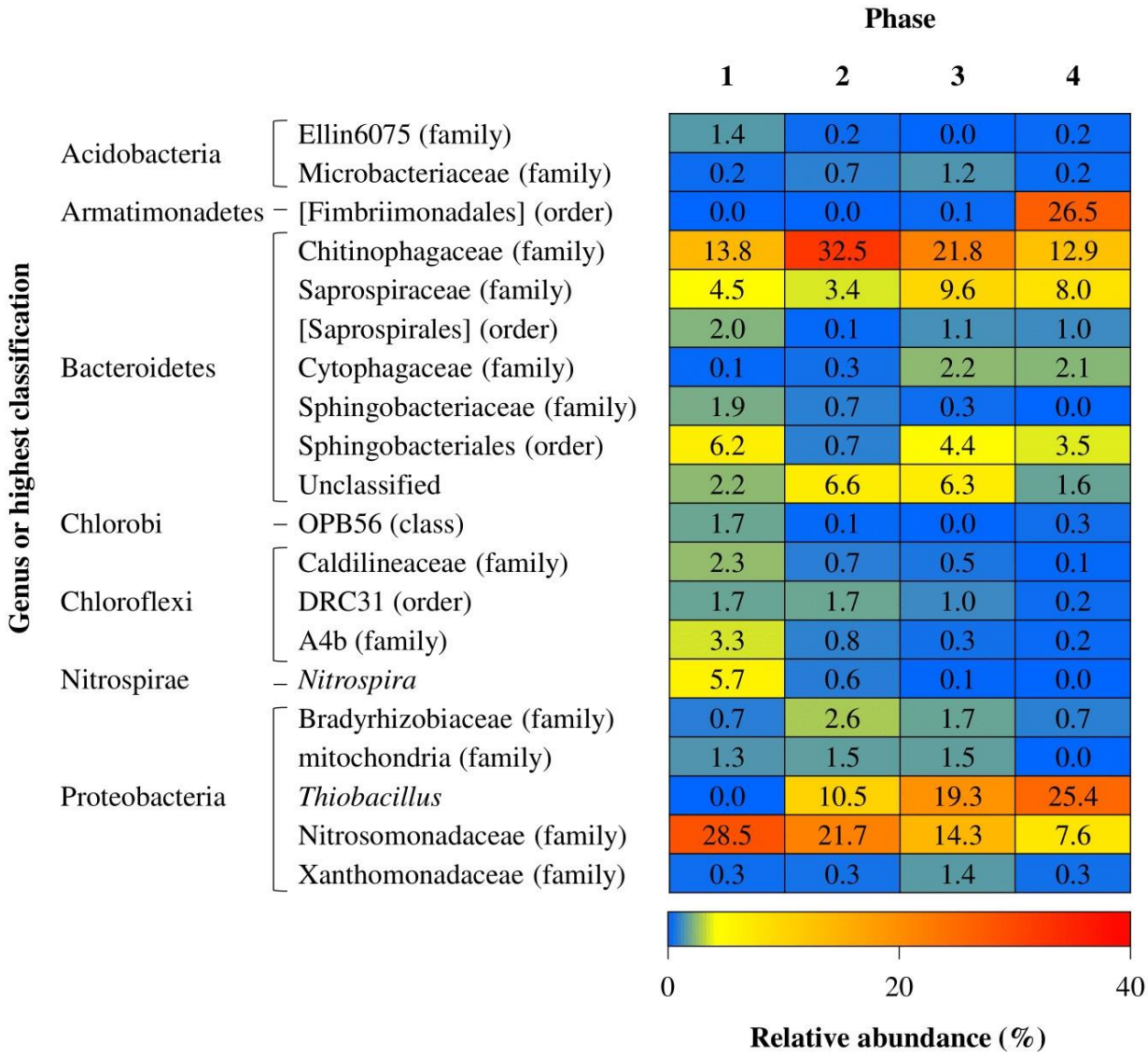


Figure 3-11. Heat map of the relative abundance of the genera which was present more than 1% in the sludge taken from the reactor at the end of each phase at least once. The indicator on the lower denotes the relationship between the relative abundance of each genus and the color range.

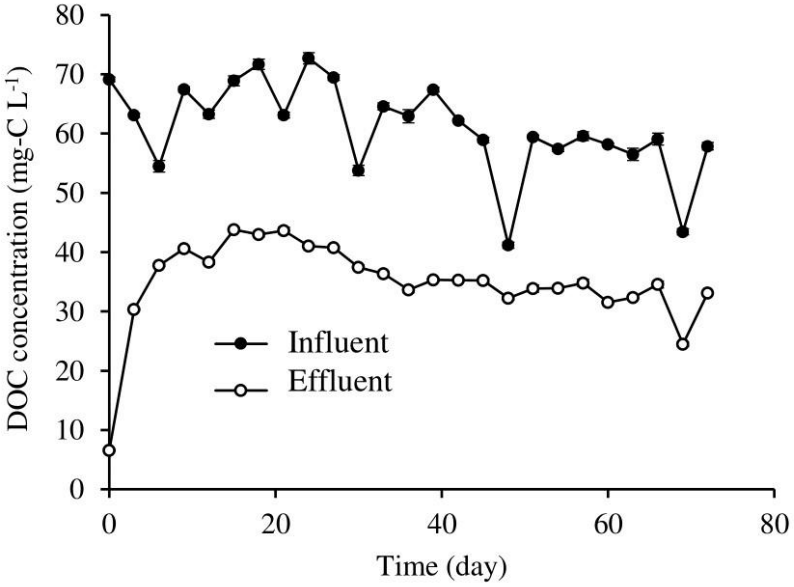


Figure 3-12. Time course of dissolved organic carbon (DOC) concentration of influent and effluent.

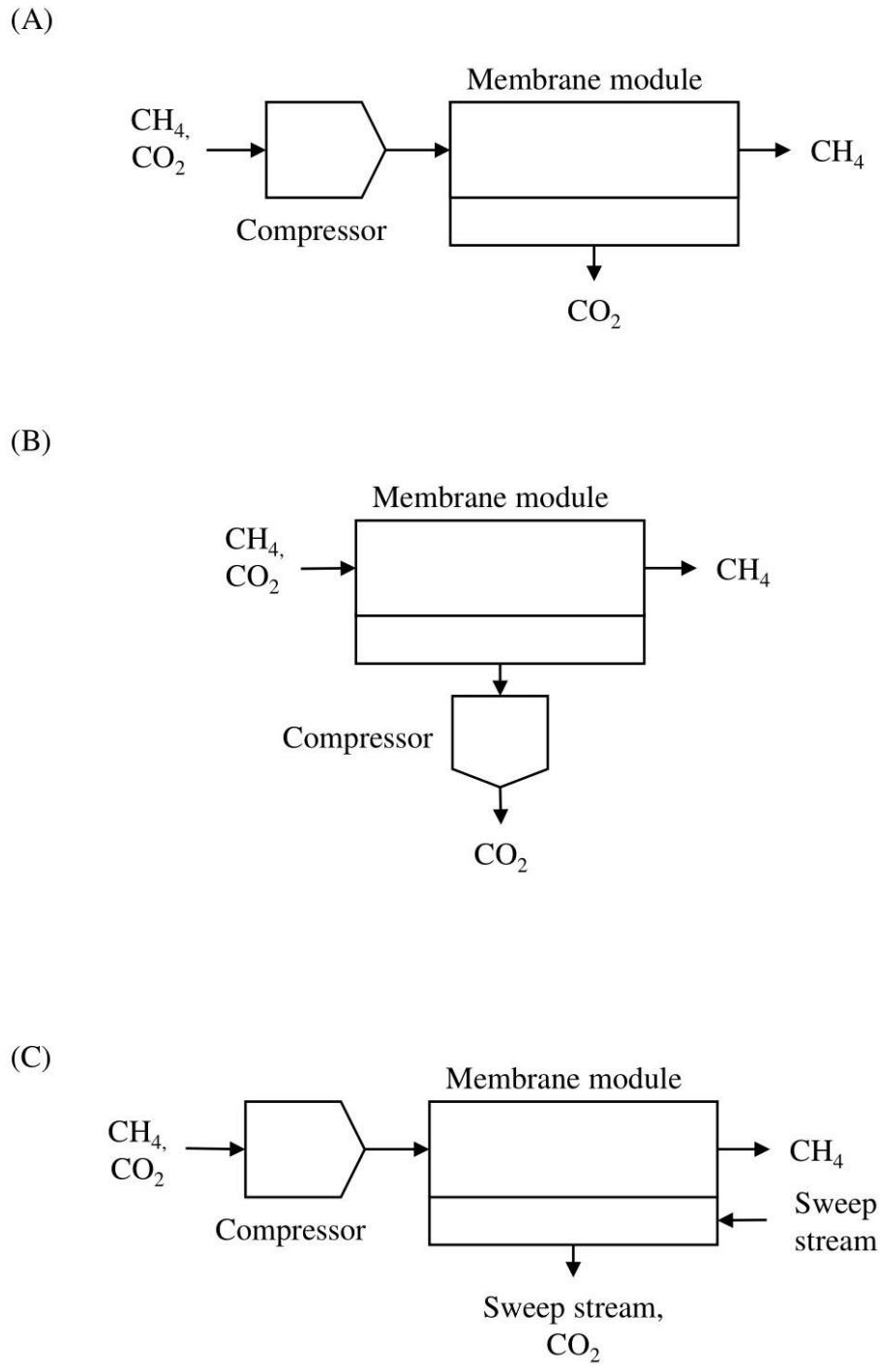


Figure 3-13. Single-stage configuration of a membrane system for CO_2 separation (Baena-Moreno et al., 2020, modified). A: Feed compression; B: Vacuum in the permeate side; C: Sweep stream configuration.

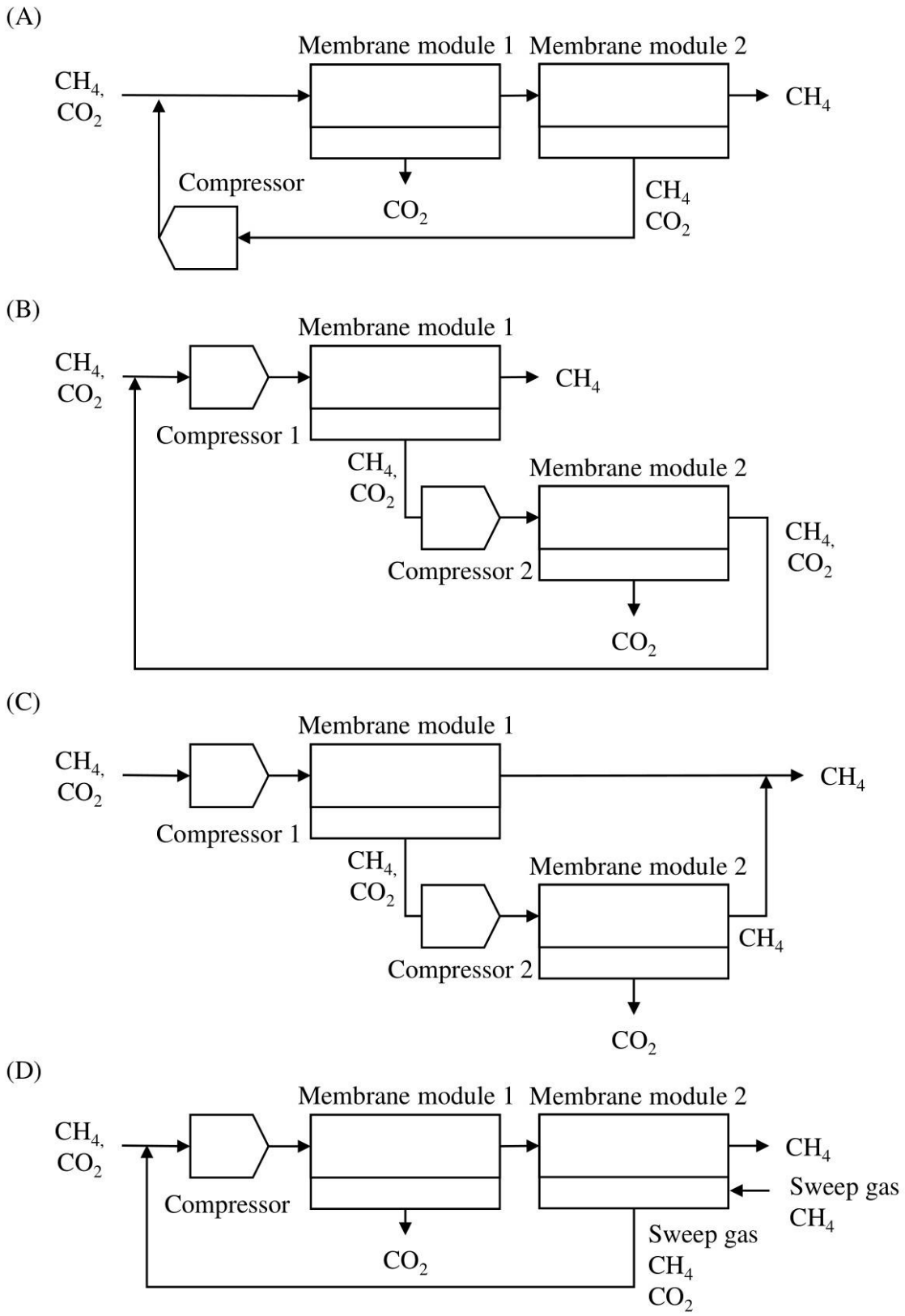


Figure 3-14. Two-stage configuration of a membrane system for CO_2 separation (Chen et al., 2015; Baena-Moreno et al., 2020; modified). A: Second membrane module and permeate recirculation; B: Second membrane module, a retentate recirculation and two compressors; C: Without recirculation of the second membrane module rretentate; D: With sweep gas use and a second membrane module with permeate recirculation.

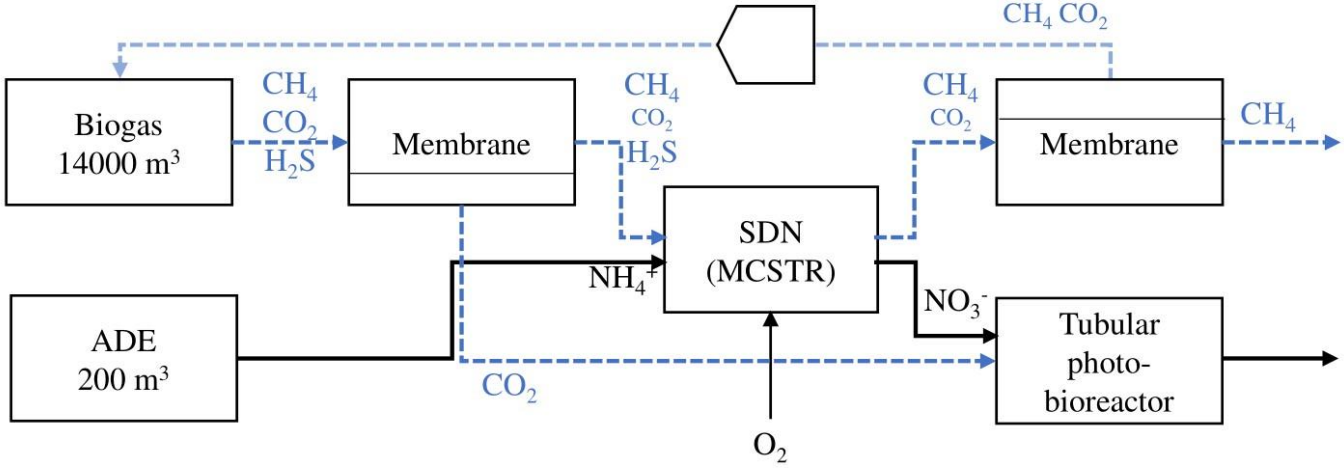


Figure 3-15. SDN treatment of biogas and ADE combined with CO_2 removal by two-step membrane separation. Dotted lines are the gas phase, and solid lines are the liquid phase.

Chapter 4

Evaluation of treated ADE usability for microalgae culture medium without dilution

4.1. Introduction

Microalgae (microscopic algae), a diverse group of photosynthetic organisms, grows using only light, CO_2, water, and nutrients. They live in various environments such as lakes, rivers, oceans, soil, and play an essential role as a primary producer of the ecosystem. Also, microalgae have a high potential for industrial application in human society due to the rapid growth rate and various bioproducts production (Brennan and Owende, 2010). Theoretical yield of microalgae was estimated as about 77 g-dry weight (DW) m^{-2} d^{-1} (280 ton ha^{-1} $year^{-1}$) with 8–10% of solar-to-product energy conversion efficiency, which is much higher than traditional C3 crop and wildland plants with less than 0.1% of conversion efficiency (Melis, 2009). The high lipid content of microalgae, usually in the range of 20–50% and can reach 80% (Sun et al., 2018), is preferable for biofuel production. The cell also contains high-value ingredients such as polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants. Therefore, microalgae have been used as protein-rich food from ancient times, and also the application has been expanded to functional feeds, nutrient supplements, coloring agents, cosmetics, and biopharmaceuticals over the last few decades (Khan et al., 2018).

Microalgae-based wastewater treatment has been developed over 60 years since it was proposed by Oswald et al. (1957). Due to the fast growth rate and a relatively high tolerance for severe environments, microalgae can effectively uptake nutrients from wastewater and produce valuable biomass. Microalgal cultivation has already been applied to both pilot- and full-scale treatments of various anaerobic digestion effluent (ADE) containing high concentrations of nitrogen and phosphorous. For example, pig-waste ADE was treated in a 23.6-m^2 raceway pond with 84%–91% NH_4^+-N and 84%–87% PO_4^{3-}-P removal rates, and *Arthrospira* sp. was produced (Olguín et al., 2003). *Arthrospira platensis* was cultivated in a 0.71-m^2 high-rate algal pond (HRAP) using ADE from sago

starch factory wastewater, and 99% NH_4^+-N was removed from ADE (Phang et al., 2000). Park and Craggs (2011) reported treatment of ADE derived from a wastewater sludge treatment facility using a microalgal–bacterial consortium in HRAP with CO_2 supply. Then, 24.7 g VSS m^{-2} d^{-1} of high biomass productivity and maximum removal of 84.5% NH_4^+ were achieved, and it was scaled up to a 5-ha full-scale plant in New Zealand (Craggs et al., 2014). However, in the microalgal cultivation using ADE, the need to dilute the ADE with a large amount of freshwater has been a serious issue. ADE usually contains 500–3,000 mg N L^{-1} of total ammonium nitrogen (TAN) (Xia and Murphy, 2016), which takes two forms, free ammonia (NH_3) and ammonium ions (NH_4^+), depending on the pH, as follows:

$$NH_3 + H_2O \rightleftarrows NH_4^+ + OH^- \text{ (pKa = 9.25).} \quad (4\text{-}1)$$

Since NH_3 severely inhibits microalgal growth, most microalgal cultivation using ADE, including the reports mentioned above, have been achieved by a 2- to 50-fold dilution of the ADE (Olguín et al., 2003; de Godos et al., 2009; Kumar et al., 2010; Wang et al., 2010b; Park and Craggs, 2011; Lee et al., 2015; Xia and Murphy, 2016). As an alternative of fresh clean water, although other water sources, such as seawater (Sepúlveda et al., 2015) and other wastewater with low TAN concentration (e.g. domestic wastewater (Dickinson et al., 2015) and secondary effluent (Bohutskyi et al., 2016)) can be used, the areas near other wastewater treatment facilities were limited, and the composition of wastewater must be carefully considered to avoid harmful contamination into the microalgae culture, particularly for advanced use of microalgae. Using seawater is suitable for seawater species but not for freshwater species.

TAN can be oxidized via nitrification by nitrifying bacteria, producing NO_3^-, which is harmless to microalgae. Thus, it is expected that ADE treated by simultaneous desulfurization–nitrification (SDN) would enable the direct use of ADE in microalgal cultivation without requiring dilution. In chapters 2–3, stable SDN treatment in a single reactor was confirmed to develop the proposed coupling process of SDN and microalgal cultivation. Meanwhile, microalgal productivity under using ADE treated by SDN remains unknown. Praveen et al. (2018) attempted to cultivate

microalgae using nitrified ADE and obtained 97% nitrogen removal efficiency. However, they treated ADE after diluting it 3–20-fold with municipal wastewater; therefore, the suitability of nitrified, undiluted ADE for microalgal cultivation has not been demonstrated. There is the possibility that microalgal growth will be inhibited by high concentrations of ADE components associated with microalgal growth other than NH_3 under a supply of undiluted ADE. Moreover, concentrations of some components may change through SDN, and cause inhibition or improvement of microalgal growth. Therefore, the present study was conducted focusing on microalgal cultivation using ADE treated by the SDN process to evaluate the suitability of the proposed coupling process.

Chlorella sorokiniana was used in the present study. The genus *Chlorella* is a typical unicellular green microalga widely distributed in the hydrosphere. *Chlorella* having a simple cell cycle, high growth rate (Masojídek and Torzillo, 2008) and high protein and lipid contents (Muys et al., 2020) has been studied intensively as a model microorganism for plant physiology, algal test systems, and mass cultivations (Krienitz, 2009). Its commercial production for food and feed began in the early 1960s, and, nowadays, it was expanded to 6600 tons-DW per year of global production (Muys et al., 2020). Also, since its high growth rate, *Chlorella* has long been used for wastewater treatment (Wang et al., 2010a; Kobayashi et al., 2013; González-Camejo et al., 2018).

First, a composition of ADE treated by the SDN process was investigated comprehensively. Second, the nitrogen utilization characteristics, the NO_3^- usability and NH_3 tolerance of *Chlorella sorokiniana* NIES-2073 were evaluated. Third, the productivity of *C. sorokiniana* using ADE treated by SDN was evaluated compared with using untreated ADE with/without dilution.

4.2. Materials and Methods

4.2.1. Inoculum and pre-culture condition

Chlorella sorokiniana NIES-2173 was purchased from the National Institute for Environmental Studies (NIES), Tsukuba, Japan. In the pre-cultivation, C-medium was used with/without 0.6-g C L^{-1} of $NaHCO_3$ which is the same condition as the experimental condition. The

500-mL conical flask was used as a container. Cultivation conditions were 200–300 mL of effective volume, 25°C of temperature, continuous aeration, and 150 μmol photons m^{-2} s^{-1} continuous light illumination. Cells in the exponential growth stage were recovered for each experiment. C-medium (Ichimura, 1971) consisted of 500-mg Tris (hydroxy-methyl) aminomethane, 150-mg $Ca(NO_3)_2 \cdot 4H_2O$, 100-mg KNO_3, 50-mg β-Na_2 glycerophosphate·$5H_2O$, 40-mg $MgSO_4 \cdot 7H_2O$, 100-μg vitamin B12, 100-μg biotin, 10-mg thiamine HCl, and 3-mL PIV trace metals solution (Provasoli and Pintner, 1959): 1-g Na_2 EDTA·$2H_2O$, 200-mg $FeCl_3 \cdot 6H_2O$, 36-mg $MnCl_2 \cdot 4H_2O$, 10.4-mg $ZnCl_2$, 4-mg $CoCl_2 \cdot 6H_2O$, 2.5-mg $NaMoO_4 \cdot 2H_2O$, per liter. The calculated concentrations of NO_3^-, PO_4^{3-}, and SO_4^{2-} were 32 mg-N L^{-1}, 7 mg-P L^{-1}, and 5 mg-S L^{-1}, respectively, and the N/P molar ratio was 10, in this medium. The salinity was changed from 0.02% to 0.22%by the addition of $NaHCO_3$ to the medium.

4.2.2. Compositional analysis of effluent from SDN process

As the SDN influent and effluent, the influents and effluents of a sequencing batch reactor (SBR) in Phase 4 with 128 mg-S L^{-1} d^{-1} of S^{2-} loading rate (SLR) in chapter 2 and a continuous stirred tank membrane reactor (CSTMR) in Phase 4 with 100 mg-S L^{-1} d^{-1} in chapter 3 were used. These were filtered through a 0.45-μm pore size glass fiber filter (GC-50; Advantec, Japan) and stored in a freezer at −30°C until use for the composition analysis and the latter experiment (microalgal cultivation). For the composition analysis, the concentrations of PO_4^{3-} and dissolved metals and the salinity in the influent and effluent were analyzed in addition to nitrogen, sulfur, and dissolved organic carbon (DOC) compounds concentrations previously reported in chapter 2.

4.2.3. Microalgal cultivation under different inorganic nitrogen source and different NH_3 concentration

First, *C. sorokiniana* was cultivated using C-medium with changes of nitrogen source to NH_4^+, NO_2^-, and NO_3^- and concentration to 33, 100, 500, 1000 mg-N L^{-1}. Instead of $Ca(NO_3)_2 \cdot 4H_2O$ and KNO_3 which are original components of C-medium, 93-mg L^{-1} $CaCl_2 \cdot 2H_2O$, 74-mg L^{-1} KCl, and different concentrations of NH_4Cl, $NaNO_3$, or $NaNO_2$ were used. The pH was adjusted to 7.5 by

adding 4.8 g L^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and NaOH solution. After the pH adjustment, each medium was sterilized by filtration using a 0.22-μm pore size syringe-driven filter unit (Millex GV; Merck Millipore, USA). 10-mL glass vials with silicone stopper were used as containers with 5 mL of effective volume. The initial call density of every vial was adjusted to 0.028 ± 0.001 of optical density at 750 nm (OD_{750}). Cultivation temperature and light intensity were 25±1ºC and 150 μmol photons m^{-2} s^{-1}, respectively. During the experimental period, the optical density at 680 nm (OD_{680}) was periodically measured after mixing culture thoroughly by inversion every 7–9 hours. The silicone stopper was removed once per day to allow air exchange.

Next, *C. sorokiniana* was cultivated using C-medium with different NH_3 concentrations (0–3.6 mM). Instead of NO_3^- of 32 mg-N L^{-1}, $NaHCO_3$ of 0.6 g-C L^{-1} and nitrogen of 941-mg-N L^{-1} (67 mM) were added to C-medium with different ratios of KNO_3 and NH_4Cl (NO_3^--N/NH_4^+-N: 67/0, 50/17, 34/34, 17/50, and 0/67 mM). The pH was adjusted to 8.0 ± 0.1 by adding 4.8 g L^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and NaOH solution. These TAN concentrations and pH values resulted in NH_3 concentrations of 0, 0.9, 1.8, 2.7, and 3.6 mM (a total of five conditions, with three to four replicates). After the pH adjustment, each medium was sterilized by filtration using a 0.22-μm pore size syringe-driven filter unit (Millex GV; Merck Millipore, USA). The twelve-well microplates were used as the cultivation containers with 2-mL effective volume. The initial cell density was adjusted to 0.021 ± 0.001 of OD_{750}. The temperature and the illumination condition were 25ºC±1 ºC and 150-μmol photons m^{-2} s^{-1}, respectively. During the experimental period, the OD_{750} was periodically measured after mixing the culture by pipetting every 8 hours. The pH value and TAN, NO_3^-, and NO_2^- concentrations were analyzed before and after cultivation.

4.2.4. Microalgal cultivation using undiluted ADE treated by SDN process

Following different types of media were used for cultivation of *C. sorokiniana*: C-medium, untreated ADE with a 1-, 3-, 6-, or 10-fold dilution, and ADE treated by nitrification–desulfurization (a total of six conditions performed in triplicate). For the conditions using ADE untreated/treated by SDN, the influent and the effluent of the SBR in chapter 2 were respectively used as the medium after

the addition of $MgSO_4 \cdot 7H_2O$ and PIV metals solution with the same concentration with C-medium. Each medium was supplemented with 0.6-g-C L^{-1} $NaHCO_3$, and the pH was adjusted to 7.5 by adding HCl solution. These were then sterilized by filtration using a 0.22-μm pore size syringe-driven filter unit (Millex GV; Merck Millipore, USA). As containers, 10-mL glass vials were used with a silicone stopper. The effective volume was 5 mL. The cultivation temperature and the illumination condition were 25±1ºC and 150 μmol photons m^{-2} s^{-1}, respectively. The cultures were mixed thoroughly by inversion every 12 h, and the OD_{750} was measured each time. The silicone stoppers were removed every 24 h for air exchange of headspace. The pH value and nutrient (TAN, NO_3^-, NO_2^-, PO_4^{3-}) concentrations were measured before and after cultivation.

4.2.5. Analytical methods and calculations

The optical densities of culture in the NH_3 tolerance test and the other experiments were measured using a microplate reader (EPOCH 2; BioTek, USA) and a spectrophotometer (DR2800; HACH, USA), respectively. The pH was measured using a compact pH meter (LAQUAtwin-pH-22B; Horiba, Japan) or a desktop pH meter (SevenCompact pH/Ion meter S220; Mettler Toledo, USA). Nutrient (TAN, NO_3^-, NO_2^-, and PO_4^{3-}) concentrations were measured using an HPLC mentioned in section 3.2.2. in chapter 3. Dissolved metals concentrations and salinity were determined by inductively coupled plasma spectroscopy (ICPS-7000ver2.1; Shimadzu, Japan) and a digital salt meter (Conductivity Method, ES-421; Aatago, Japan), respectively.

The specific growth rate of *C. sorokiniana* was calculated using Equation (4-2).

$$\mu = \ln (X_1 / X_2) / (t_2 - t_1) \quad (4\text{-}2)$$

where μ is specific growth rate (d^{-1}) and X_1 and X_2 are the OD_{680} or OD_{750} at time t_1 and t_2 (d), respectively. The NH_3 ratio in TAN (%) was calculated using Equation (4-3) (Anthonisen et al., 1976):

$$NH_3(\%) = 10^{pH} / (e^{(6344/T)} + 10^{pH}) \times 100 \quad (4\text{-}3)$$

where T is the temperature (K). The half-maximal effective concentration (EC_{50}) of NH_3 on microalgae was determined by fitting microalgal specific growth rates at the different NH_3 concentrations to the following four-parameter logistic curve-regression using SigmaPlot 11.0

software (Systat Software, UK):

$y = min + (max - min) / (1 + (x / EC_{50})^{-Hillslope}) \times 100$

where min is the bottom of the curve, max is the top of the curve, and *Hillslope* is the slope of the curve at its midpoint.

4.3. Results and Discussion

4.3.1. Composition of effluent from SDN process

Table 4-1 shows the composition of the influent and effluent of the SDN reactors, SBR (Phase 4, chapter 2) and CSTMR (Phase 4, chapter 3). Through the SDN treatment in both reactors, TAN was completely converted to NO_3^-. In the SBR under the supply of 384 mg-S L^{-1} of S^{2-}, S^{2-} was removed at 100% efficiency. 40% of removed S^{2-} was converted to SO_4^{2-}, leading to an increase in SO_4^{2-} concentration from 25 mg-S L^{-1} to 183 mg-S L^{-1}. Because H_2S was not detected from the exhaust gas, and white precipitates were observed attached to the reactor, elemental sulfur may have been produced from a part of S^{2-}. On the other hand, in the MSCSTR, SO_4^{2-} concentration increased from 81 mg-S L^{-1} to 378 mg-S L^{-1} as the result of the supply of H_2S gas with 100 mg-S L^{-1} d^{-1} and 3-days hydraulic retention time (HRT), indicating supplied H_2S was completely oxidized to SO_4^{2-}. This different trend between the two reactors was probably caused by the microbial composition difference (section 3.3.4., chapter 3). The concentration of SO_4^{2-} is generally 0.3–1.6 mg-S L^{-1} in the soil/freshwater environments where microalgae grow (Bochenek et al., 2013), and 3–160 mg S L^{-1} in microalgal synthetic medium (Mera et al., 2016). Therefore, the SO_4^{2-} concentration in influent and effluent of both SDN reactors are probably sufficient for microalgal cultivation. Also, because SO_4^{2-} does not inhibit the freshwater microalgae *Chlamydomonas moewusii* even at concentrations of more than 600 mg-S L^{-1} (Mera et al., 2016), increased SO_4^{2-} will not inhibit microalgae. The PO_4^{3-} concentration of the effluent of the SBR fluctuated in the range of 15 to 101 mg-P L^{-1}, with an average of 58 mg L^{-1}, and tended to be lower than the average influent concentration of 119±11 mg-P L^{-1}. Whereas, PO_4^{3-} concentration did not decrease in the CSTMR. PO_4^{3-} easily precipitates in the

presence of some components that exist in ADE, such as TAN, Mg, Al, Ca, and Fe, particularly under alkaline pH conditions. More than 50% of Al, Ca, and Fe were also removed in this experiment, as described below. In particular, Ca does not precipitate with S^{2-}. Therefore, the precipitation of PO_4^{3-} with these components may have caused through the SDN treatment. Other than precipitation, aerobic sludge, such as activated sludge and nitrifying sludge, has the potential to remove phosphorus for the assimilation it into the cells of microbes. Additionally, uptake of PO_4^{3-} by polyphosphate accumulating organisms can be occurred using organic carbon under an anoxic environment (Tayà et al., 2011). In contrast to the SBR operation having anoxic periods (settle and discharge periods), CSTMR which is a continuous aeration operation might have mitigated PO_4^{3-} removal. This remaining of PO_4^{3-} in the SDN effluent contributes to microalgal productivity. The molar ratios of N/P of the effluent of SBR and CSTMR were 53± 42 and 33 ± 2, respectively. Microalgae can live in freshwater environments with a wide range of N/P molar ratios, from 8 to 45, by changing their cellular composition according to the surrounding nutrients in their environment, but a high enough level of phosphorus (N/P< 22) is required to maintain a high growth rate (Hecky et al., 1993; Beuckels et al., 2015). Therefore, especially in the continuous algal cultivation, the addition of PO_4^{3-} to ADE that has been treated with SDN is probably required to adjust the N/P molar ratio of culture to <22. Note that the N/P ratio of ADE varies depending on the original substrate type and AD treatment methods, therefore, the required amount of PO_4^{3-} addition also be changed depending on the ADE composition. The salinity of ADE increased from 0.35% to 0.66% and from 0.37% to 0.54% in SDN treatment using SBR and CSTMR, respecvitely, with NaOH addition which was to maintain the reactor pH decreasing by oxidation of S^{2-} and NH_4^+. There is a report about the reduction of the final biomass concentration of *Chlorella vulgaris* by approximately 30% under 0.3% salinity, in 24-day batch cultivation (Kebeish et al., 2014). This increase in salinity may negatively affect microalgal productivity depending on the salinity tolerance of the algal strains used.

The concentration of most metals decreased in the SDN reactor (SBR in chapter 2) (Table 4-2). Microbes can remove metals by both the assimilation into cells and the sorption to flocs (Üstün

2009). Additionally, several metals (Al, Mn, Zn, Fe, Ni, Cd, Sn, Pb, Cu, Hg, Ag, Pt, and Au) can easily precipitate in the presence of S^{2-} (Lewis, 2010). It was also possible to precipitate with phosphorous removed at the same time. This decrease in metals concentration in an SDN reactor leads to reduce the effects of inhibitory metals (Al, Co, Ni, Cu, Cd, and Pb) in microalgal cultivation, although some essential metals (Mg, K, Ca, Fe, Mn, and Zn) should be supplemented if concentrations are insufficient. Additionally, from a commercial perspective, the removal of metals by an SDN reactor may effectively mitigate the accumulation of heavy metals which are toxic to humans in produced microalgae. It has been raised as an issue that toxic heavy metals detected in *Arthrospira platensis,* at a higher concentration than the upper limit of the acceptable range for human intake, following the contamination of a cultivation pond with environmental pollutants (Boudene et al., 1975). Especially in the microalgal cultivation using ADE, the accumulation of toxic metals in produced microalgae should be carefully avoided for the commercialization of microalgae to high-added-value products such as feeds and food supplements (Chamorro-Cevallos and Barrón, 2007).

4.3.2. Usability of NO_3^- and NH_3 tolerance of microalgae

To evaluate the NO_3^- usability of *C. sorokiniana* NIES-2173, the strain was cultivated using different inorganic nitrogen: NH_4^+, NO_2^-, and NO_3^-. After 56-h cultivation, the OD_{680} of each condition was higher in the order of conditions using NH_4^+ (0.104–0.125), NO_3^- (0.091–0.107), and NO_2^- (0.031–0.099) (Figure 4-1). And, it tended to decrease with an increase in concentration regardless of the nitrogen species. In particular, the OD_{680} drastically decreased after 16 h in the condition using NO_2^- with 1000 mg-N L^{-1}, indicating that microalgae died of strong NO_2^- inhibition. Table 4-3 shows the specific growth rate in each condition calculated using growth data for the initial 25 hours, except the value of the condition using NO_2^- of 1000 mg L^{-1}. As the same with final OD_{680}, the specific growth rate tended to decrease in conditions using NO_3^- and especially NO_2^- with higher concentrations. Microalgae generally preferentially assimilate NH_4^+ to avoid energy consumption in the assimilation process of NO_3^- or NO_2^- (incorporation into cells and reduction to NH_4^+ for assimilation) (Collos and Harrison, 2014). Also, it has been suggested that non-dissociated NH_3

coexisting with NH_4^+ is produced inside the cell during the reduction process of NO_3^- and NO_2^- and inhibits microalgae when the concentration is high (Collos & Harrison, 2014). This may be the reason for the lower microalgal growth rate under higher nitrogen concentration. Regarding strong inhibition of NO_2^-, because it was reported that NO_2^- inhibits Photosystem II (Zhang et al., 2017), it was considered that the photosynthetic activity of the used strain also dramatically reduced at the high concentration by the same mechanism although inhibition may be suppressed at low concentration by rapid assimilation inside the cell. Since NO_2^- is an intermediate of nitrification reaction, partial nitrification must be avoided in the SDN to utilize effluent for the microalgal cultivation.

In the evaluation of NH_3 tolerance of microalgae, through 56-h cultivation, the OD_{750} increased to from 0.097 ± 0.018 to 0.030 ± 0.021 under 0-, 0.9-, 1.8-, 2.7-, and 3.6-mM initial NH_3 concentration (Figure 4-2, Table 4-4). It tended to decrease with initial NH_3 concentration. The pH increased to 9.47 maximum following microalgal growth with inorganic carbon consumption. In the conditions with 0.9-, 1.8-, 2.7-, and 3.6-mM initial NH_3 concentration, the TAN concentration was decreased by 21%–78%. NH_3 volatilization was considered as a reason for the reduction. This is because the NO_3^- concentration was almost unchanged in all conditions, including the 0-mM NH_3 concentration condition using only NO_3^- even in where the highest microalgal growth was obtained, indicating that both nitrification and assimilation into microalgae biomass of supplied TAN were almost not occurred. The specific growth rate was calculated to 1.85 ± 0.22, 1.32 ± 0.53, 0.77 ± 0.37, −0.14 ± 0.37, and −0.16 ± 0.28 d^{-1} in conditions with the initial NH_3 concentrations of 0, 0.9, 1.8, 2.7, and 3.6 mM, respectively, based on the variation of OD_{750} in the logarithmic growth period (8–24 h) (Table 4-4); that is, a decreasing trend with the increase in initial NH_3 concentration was shown. Two reactions leading to the breakdown of photosystem II (PSII) function have been mainly considered as the reason for NH_3 inhibition to the microalgal growth: (1) by diminishing the proton gradient across the thylakoid membrane and suppressing adenosine triphosphate (ATP) production, which is necessary for repairing photo-damaged PSII (McCarty, 1969; Gutierrez et al., 2016); and (2) by ligation to the organometallic reactor core in the D1 subunit of PSII which is essential for its correct

functioning (Britt et al., 2004; Gutierrez et al., 2016). These inhibitory mechanisms associated with NH_3 probably led to the decreased growth of *C. sorokiniana* NIES-2173 we observed. The correct functioning of PSII tends to be maintained until the NH_3 concentration exceeds a certain concentration and the mechanisms mentioned above take effect, therefore the NH_3 inhibition level can be easily fitted to a sigmoid regression curve to obtain the EC_{50} (Azov and Goldman, 1982). Based on this fitting regression, the EC_{50} of NH_3 for the *C. sorokiniana* NIES-2173 used in the present study was 1.6 mM ($p< 0.01$, Figure 4-3). This value falls within the range of EC_{50} for other species from 0.003 to 3.3 mM, both reported by Collos & Harrison (2014) and calculated from other studies (Figure 4-3; Azov and Goldman, 1982; Belkin and Boussiba, 1991; Tan et al., 2016). Therefore, *C. sorokiniana* NIES-2173 is reasonable to be used as a representative species to validate SDN treatments to avoid NH_3 inhibition.

4.3.3. Usability of treated ADE without dilution for the microalgal cultivation

C. sorokiniana NIES-2173 was cultivated in batch mode using C-medium, untreated ADE with 1-, 3-, 6- and 10-fold dilutions, and ADE treated by SDN (Figure 4-4). The specific growth rates within the first two days were almost the same at 0.43–0.57 d^{-1} for all conditions except untreated ADE without dilution (0.32 d^{-1}) (Table 4-5). The specific growth rate for the condition using untreated ADE without dilution was lower by 44% than that under using C-medium, probably because of the highest initial NH_3 concentration of 0.91 mM. Therefore, the introduction of SDN treatment is useful for microalgal cultivation using ADE without dilution. In this connection, although a higher growth rate of microalgae is generally obtained using TAN more than using NO_3^- as shown in section 4.3.2. because TAN assimilation requires less energy than NO_3^- assimilation (Sanz-Luque et al., 2015), the specific growth rate did not decrease in C-medium and treated ADE using NO_3^- compared with untreated and diluted ADE using NH_4^+. There is a possibility that microalgal growth was improved by increasing contaminant concentrations (e.g., DOC, SO_4^{2-}) or decreasing toxicity of metals via SDN treatment, apart from the suppression of NH_3 inhibition. However, the effect of salinity, observed negligible in the present study, might differ if other microalgae species are used. The salinity tolerance

of *Chlorella* spp. and *Chlorococcum* spp. that are commonly found in terrestrial habitats have been reported at 0.9%–5% of EC_{50} which is higher than that of other freshwater microalgae (EC_{50}: 0.3%–1.8%) (Kim et al., 2016; von Alvensleben et al., 2016; Figler et al., 2019). Therefore, it may be necessary to select species that exhibit enough salinity tolerance for microalgal cultivation using ADE treated by SDN.

In the latter phase of the growth curve (Figure 4-4), the microalgal growth rate in all conditions using untreated ADE, which was initially almost the same as with the other medium conditions, decreased gradually. The final OD_{750} was 40% to 88% lower than that in conditions using C-medium. The pH increased to 8.9–11.2 in all conditions during the cultivation period (Table 4-5), leading to an increase in 0.08–0.85 mM of the NH_3 concentration to 2.12–13.2 mM (Table 4-5) and inhibition of microalgal growth in conditions using untreated ADE. However, the same final OD_{750} of that using C-medium condition was achieved using ADE treated by SDN.

At the end of the experiment, both nitrogen and phosphate remained, in all conditions, more than 20.0-mg-N L^{-1} and 3.5-mg-P L^{-1}, respectively (Figure 4-5). The growth of *C. sorokiniana* in conditions using C-medium and treated ADE was suppressed by other parameters such as light limitation, increased pH, and lack of dissolved inorganic carbon, trace metals, and other micronutrients, rather than by nitrogen or phosphate limitation. TAN volatilization was probably reduced by using a glass tube with a silicone stopper as a cultivation container compared with the NH_3 tolerance assay using microplates which resulted in a loss of 21%–78% TAN (section 4.3.2). However, volatilization of TAN easily occurs through culture mixing, gas exchange, and/or increases in pH in the microalgal cultivation using untreated ADE. The prevention of nitrogen loss from ADE to the atmosphere should be an advantage of converting TAN to NO_3^- by nitrification treatment.

All ADE composition analysis and microalgal cultivation in the present study were conducted using ADE after freezing and thawing to unify the analysis and cultivation conditions. This freezing and thawing step might have changed the treated/untreated ADE composition; that is, by precipitation of dissolved contaminates and decomposition of organic compounds, and affected the

microalgal growth. The effect of this experimental step on microalgal growth required further confirmation in the next chapter. To avoid inhibition of growth by NH_3 in microalgal cultivation using ADE, sustaining the pH at less than 7.5 or controlling the concentration of TAN inside the reactor are also ways other than the dilution of ADE. Plus, the high microalgal growth rate may be obtained when NH_4^+ is used as a nitrogen source. However, complete agitation required to maintain uniform pH in the reactor is difficult to achieve in a large full-scale reactor (Eroglu et al., 2014). Also, control of TAN concentration is difficult because the TAN removal rate in a reactor fluctuates widely depending on the algal growth rate and environmental parameters associated with TAN volatilization efficiency, particularly outdoor cultivation (González-Camejo et al., 2018). In the present study, it was confirmed that microalgae grow well in ADE treated by SDN, as well as in a synthetic medium, even without dilution. It is considered that the proposed process can be an effective option to recover nutrients from the ADE as variable microalgal biomass that will contribute to the further spread of the AD process. Moreover, most metals were removed through the SDN process, which can mitigate the bioconcentration of toxic heavy metals from wastewater in microalgal biomass. Meanwhile, the present study also identified some problems: a higher N/P molar ratio and salinity of treated ADE than is appropriate for microalgal cultivation. The need to add PO_4^{3-} is may unavoidable, especially for continuous microalgal cultivation. The high salinity can limit various algal species that can be cultured in this process.

Table 4-1. Nitrogen, sulfur, and other compounds concentrations of anaerobic digestate before and after simultaneous desulfurization-nitrification (SDN) treatment using a sequential batch reactor (SBR) in Chapter 2 and a continuous stirred tank membrane reactor (CSTMR) in Chapter 3.

Elements	SBR in Chapter 2		CSTMR in Chapter 3	
	Influent	Effluent	Influent	Effluent
TAN-N (mg L^{-1})	856 ± 12	n.d.	802 ± 50	n.d.
NO_2^--N (mg L^{-1})	n.d.	n.d.	n.d.	n.d.
NO_3^--N (mg L^{-1})	n.d.	847 ± 54	n.d.	793 ± 13
S^{2-}-S (mg L^{-1})	384 [a]	n.d.	n.d.	n.d.
$S_2O_3^{2-}$-S (mg L^{-1})	n.d.	n.d.	n.d.	n.d.
SO_4^{2-}-S (mg L^{-1})	25 ± 1	183 ± 34 [b]	81 ± 6	378 ± 6 [d]
PO_4^{3-}-P (mg L^{-1})	119 ± 11	58 ± 38 [b]	49 ± 3	51 ± 3
DOC (mg L^{-1})	63 ± 1	81 ± 1 [d]	53 ± 1	31 ± 0 [b]
Salinity (%)	0.35 ± 0.06	0.66 ± 0.04 [c]	0.37 ± 0.01	0.54 ± 0.02 [d]

TAN: Total ammonium nitrogen; DOC: Dissolved organic carbon; n.d.: not detected. a: calculated from supplied solution concentration. Student's t-test was conducted on SO_4^{2-}, PO_4^{3-} and DOC concentrations and salinity of influent and effluent ($n \geq 3$). b: $p<0.05$, c: $p<0.005$, d: $p<0.001$.

Table 4-2. Metals concentrations of anaerobic digestate before and after simultaneous desulfurization-nitrification (SDN) treatment using a sequential batch reactor (SBR) in Chapter 2.

Essential metals for microalgae				Inhibitory metals for microalgae			
Metal	Concentration (mg L^{-1})		Change (%)	Metal	Concentration (mg L^{-1})		Change (%)
	Influent	Effluent			Influent	Effluent	
Mg	3.2	2.9	-10.5	Al	0.35	0.18	-50.1
K	31.2	39.5	26.6	Co	0.16	0.04	-73.8
Ca	19.4	5.3	-72.5	Ni	0.29	0.19	-34.4
Fe	0.34	0.10	-70.6	Cu	0.44	0.13	-70.7
Mn [a]	0.08	0.02	-71.4	Cd	0.19	0.07	-66.3
Zn [a]	0.19	0.06	-65.4	Pb	0.75	0.15	-80.5

[a] Metals which inhibit microalgae under high concentration.

Table 4-3. The specific growth rate of *Chlorella sorokiniana* NIES-2173 under using different inorganic nitrogen with different concentrations.

Nitrogen concentration (mg-N L^{-1})	Specific growth rate under using different nitrogen source (d^{-1})		
	NH_4^+	NO_2^-	NO_3^-
33.3	0.036 ± 0.001	0.026 ± 0.001	0.032 ± 0.001
100	0.034 ± 0.006	0.024 ± 0.002	0.029 ± 0.006
500	0.030 ± 0.005	0.015 ± 0.001	0.023 ± 0.005
1000	0.037 ± 0.001	—	0.020 ± 0.006

Table 4-4. Specific growth rate and final optical density (OD_{750}) of *Chlorella sorokiniana* NIES-2173 and cultivation conditions under different NH_3 concentrations.

Initial NH_3 concentration (mM) [TAN concentration (mM)]	Specific growth rate [a] (d^{-1})	Final OD_{750}	Final pH	TAN reduction (%) [Final concentration (mM)]
0 [0]	1.85 ± 0.22	0.10 ± 0.02	9.47	−
0.9 [17]	1.32 ± 0.53	0.13 ± 0.02	9.21	78 [3.7 ± 1.2]
1.8 [34]	0.77 ± 0.37	0.08 ± 0.02	8.63	69 [10.5 ± 3.6]
2.7 [50]	−0.14 ± 0.37	0.03 ± 0.01	8.01	44 [28.2 ± 3.5]
3.6 [67]	−0.16 ± 0.28	0.03 ± 0.02	7.93	21 [53.2 ± 1.2]

[a] calculated from the OD_{750} between 8 and 24 hours of the logarithmic growth period.

Table 4-5. Specific growth rate and final optical density (OD_{750}) of *Chlorella sorokiniana* NIES-2173 and cultivation conditions under different mediums.

Medium		Specific growth rate (d^{-1})	Final OD_{750}	Final pH	NH_3 concentration (mM)	
					Initial	Final
C-medium		0.57 ± 0.16	0.71 ± 0.01	10.5	0	0
Untreated anaerobic digestion effluent (ADE)	Without dilution	0.32 ± 0.04 [a]	0.08 ± 0.01 [b]	8.9	0.85	13.2
	3-fold dilution	0.43 ± 0.05	0.23 ± 0.02 [b]	9.3	0.35	7.15
	6-fold dilution	0.45 ± 0.11	0.26 ± 0.03 [b]	9.6	0.16	3.40
	10-fold dilution	0.47 ± 0.04	0.43 ± 0.03 [b]	10.1	0.08	2.12
Treated ADE		0.54 ± 0.09	0.71 ± 0.02	11.2	0	0

Dunnett's test was conducted on specific growth rate and final OD_{750} values (n=3) assuming C-medium condition was the control. a: significantly different at the 5% level, b: significantly different at the 1% level.

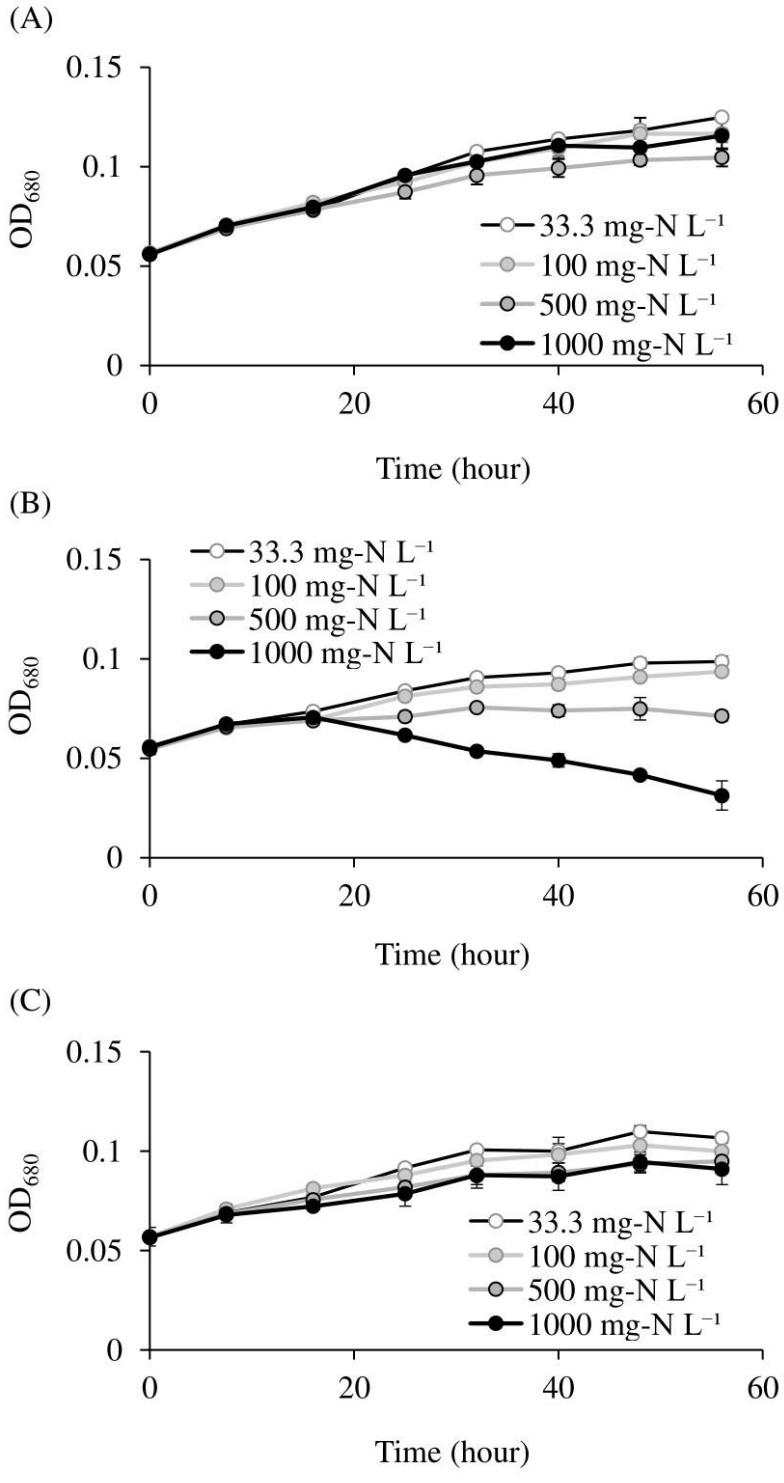


Figure 4-1. Time course of the optical density at 680 nm (DO_{680}) under using different inorganic nitrogen: NH_4^+ (A), NO_2^- (B), NO_3^- (C) with different concentration.

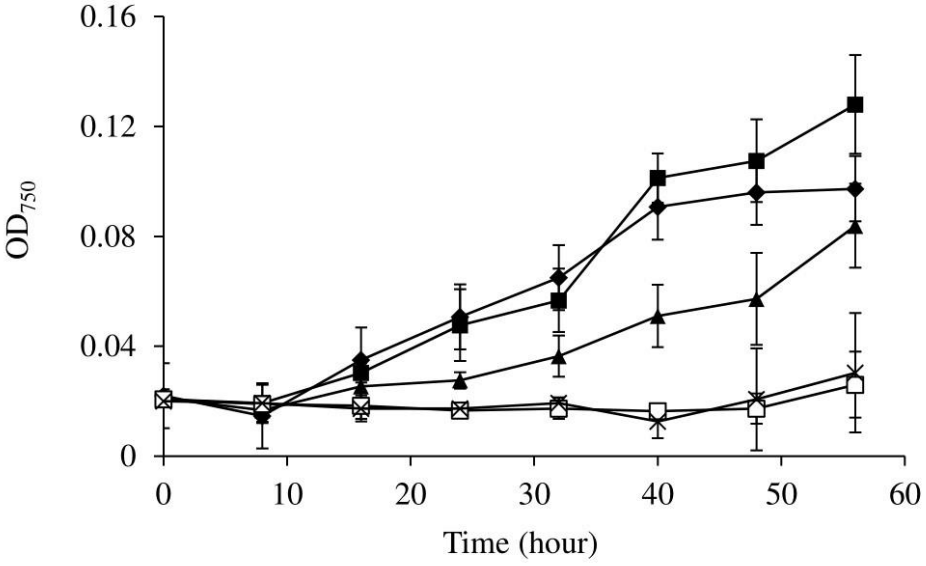


Figure 4-2. Time course of the optical density at 750 nm (OD_{750}) under different initial NH_3 concentration: 0 mM (◆), 0.9 mM (■), 1.8 mM (▲), 2.7 mM (□), and 3.6 mM (×).

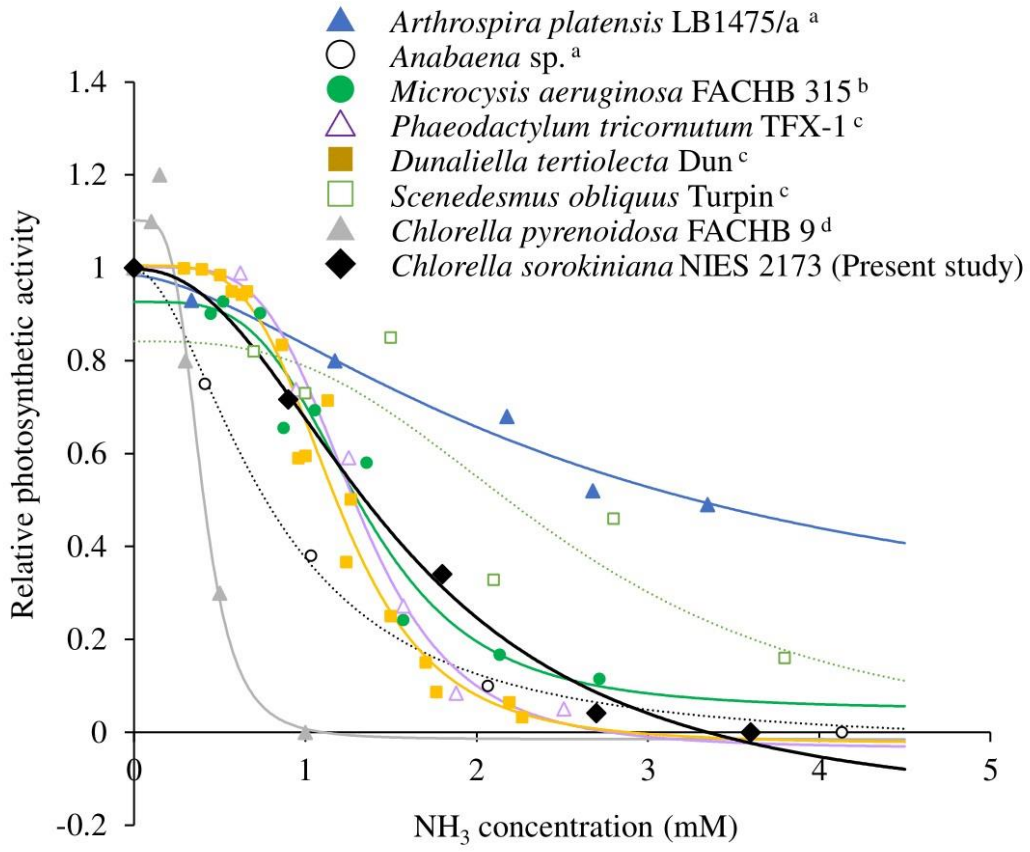


Figure 4-3. Microalgal photosynthetic activities under different NH_3 concentrations in previous and present studies. [a]: Oxygen generation rate (Belkin & Boussiva 1991), [b]: Specific growth rate (Dai et al. 2008), [c]: ^{14}C uptake rate (Azov & Goldman 1982), [d]: Specific growth rate (Tan et al 2016). Obtained 50% effective NH_3 concentration (EC_{50}) by fitting the sigmoid regression with correlation coefficient $R>0.94$ were *Arthrospira platensis* LB1475/a: 2.6 mM, *Anabaena* sp.: 0.80 mM, *Microcysis aeruginosa* FACHB 315: 0.39 mM, *Phaeodactylum tricornutum* TFX-1: 1.3 mM, *Dunaliella tertiolecta* Dun: 1.3 mM, *Scenedesmus obliquus* Turpin: 1.2 mM, *Chlorella pyrenoidosa* FACHB 9: 2.5 mM, and *Chlorella sorokiniana* NIES 2173: 1.6 mM. All obtained EC_{50} are statistically significant at $p<0.01$.

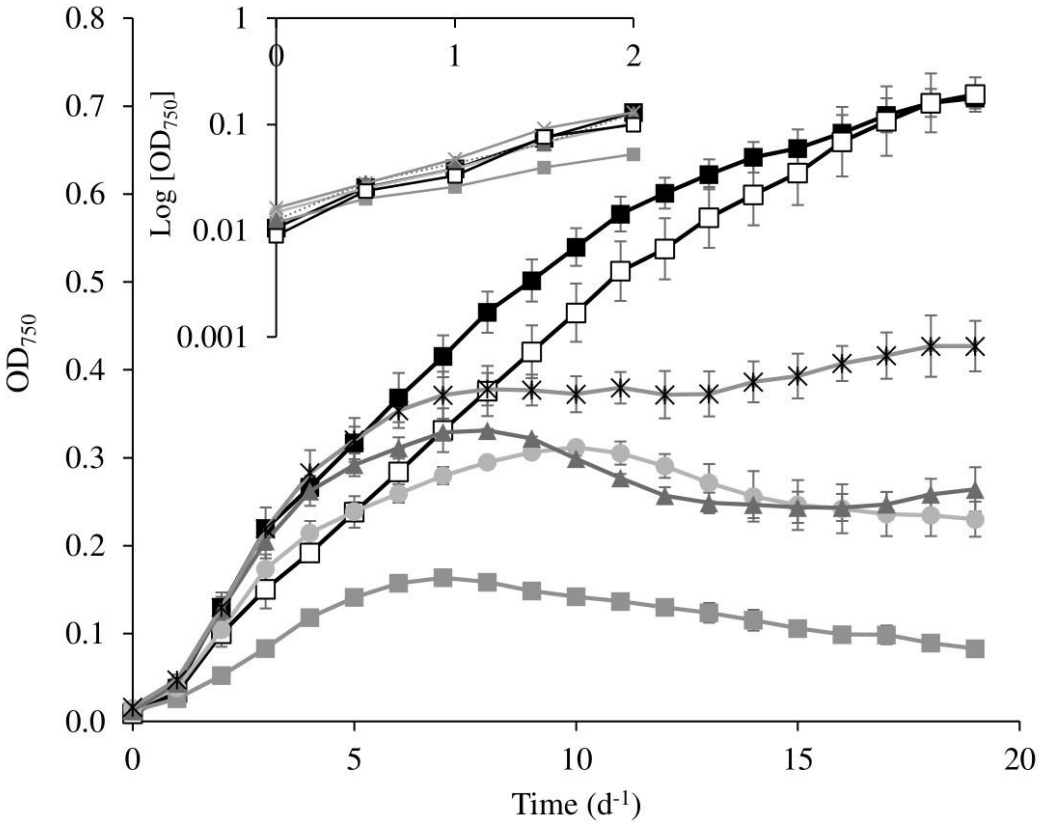


Figure 4-4. Time course of the optical density at 750 nm (OD_{750}) under using C-medium (■), 1- (without), 3-, 6-, or 10-fold diluted untreated anaerobic digestion effluent (ADE) (■, ●, ▲, ×), and ADE treated by desulfurization-nitrification (□).

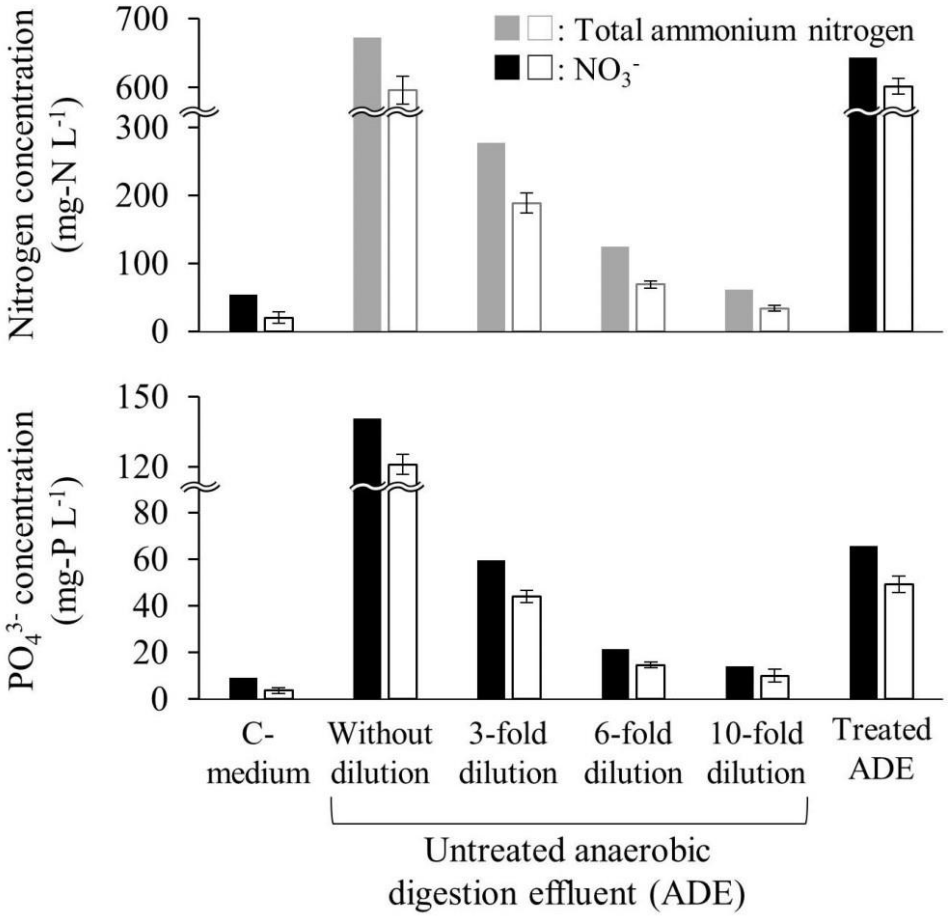


Figure 4-5. Nitrogen compounds and phosphate concentrations of the medium before (Filled bar) and after (Hollow bar) *Chlorella sorokiniana* NIES-2173 cultivation.

Chapter 5

General discussion

5.1. Achievements in chapters 2–4

The proposed novel post-treatment of anaerobic digestion (AD) consisted of SDN and microalgal cultivation process has the potential to achieve biogas and AD effluent (ADE) treatments and production of valuable microalgae, simultaneously, with fewer facilities, environmental loads, and cost. To establish this system, underlying technologies for each process, simultaneous desulfurization–nitrification (SDN) and microalgal cultivation, were developed through studies in chapters 2–4 (Figure 5-1).

In chapter 2, the SDN process was developed using an sequential batch reactor (SBR) with a long fill period operation under NaHS solution supply instead of H_2S gas. Although S^{2-} is a strong inhibitor for nitrification, it was revealed that the stable and high nitrification efficiency can be achieved under a high S^{2-} loading rate (SLR) until 128 mg-S L^{-1} d^{-1}, which is high enough to treat ADE and biogas together. It was considered that high sludge retention ability and gradual substrate supply in the SBR with a long fill period is appropriate for nitrifying bacterial acclimatization to S^{2-}.

In chapter 3, the simultaneous treatment of biogas and ADE was attempted. The batch experiment results, which is the 60% reduction of nitrification activity under 40% CO_2 supply, indicated that CO_2 removal before the SDN process is necessary. In an experiment that followed, complete SDN treatment of synthetic biogas (0.5% H_2S) without CO_2 and ADE was performed using an O_2 bubbling column and continuous stirred tank membrane reactor (CSTMR) which also operated with a high sludge retention ability and gradual substrate supply. O_2 contamination into biogas was mitigated until less than 0.4% on an average under 3.0-mg L^{-1} dissolved oxygen (DO) concentration, enabling safety biogas utilization,

In chapter 4, high productivity of *C. sorokiniana* NIES-2173 was achieved without dilution, the same as was seen with synthetic medium, indicating that this novel ADE treatment by the SDN,

without dilution, is useful for microalgal cultivation using ADE with suppression of the amount of freshwater consumption.

Demonstrations of stable SDN treatment of biogas and ADE and high microalgal productivity using undiluted SDN effluent were accomplished in the present thesis. Therefore, the feasibility of the coupling process of SDN and microalgal cultivation for biogas and effluent from AD was assessed as being high. SDN effluent had a relatively high salinity (0.54-0.66%) due to the addition of NaOH in the SDN to adjust pH. The microalgal species used in this process should be considered.

5.2. Coupling operation of SDN and microalgal cultivation and estimation of mass balance

In this section, the coupling operation of SDN and microalgal cultivation was attempted for 20 days as a trial, and the mass balance of sulfur, nitrogen, and also oxygen, carbon, and phosphorus was estimated to consider the appropriate operation condition and scale of reactors based on S^{2-} and NH_4^+ loading rates. Also, a comparison of O_2 consumed in SDN and its produced in microalgal cultivation is important for further utilization of O_2 produced in microalgal cultivation in SDN treatment.

The same ADE and nitrifying sludge with those in section 2.2.1, chapter 2 were used as a substrate and inoculum, respectively, after the same pre-treatments (Table 5-1). ADE and synthetic biogas without CO_2 (0.5 % H_2S, N_2 base instead of CH_4) were treated by an CSTMR with the same operation condition with chapter 2 written in section 3.2.3 (Figure 5-2). SLR and NH_4^+ loading rate (NLR) were 100 mg-S L^{-1} d^{-1} and 283±25 mg-N L^{-1} d^{-1}, respectively, based on the H_2S and NH_4^+ concentrations in biogas and ADE and 3-day HRT. *Chlorella sorokiniana* NIES-2173 was cultivated using an acrylic airlift photo-bioreactor with 4.5 L effective volume (Figure 5-2). As a substrate, SDN effluent was used after adding $MgSO_4$ and trace metals with a 5-time higher concentration of C-medium. Firstly, the strain was cultivated in the reactor with batch mode using SDN effluent stocked in the refrigerator for less than one week. Then, the reactor operation was changed to semi-continuous

mode. Air stored in a gas bag was continuously supplied for agitation of the culture. Reactor pH was automatically adjusted to 7.5 by pure CO_2 supply. Hydraulic retention time (HRT) and sludge retention time (SRT) were 5 days and 2 days, respectively. The reactor temperature and light intensity were 30°C and 600 μmol-photons m^{-2} s^{-1} (continuous), respectively. Further experimental details were shown in Table 5-1.

5.2.1. Biogas and ADE treatment and microalgal production in the coupling process

In the SDN treatment of synthetic biogas consisted of N_2 and H_2S, and ADE using CSTMR, both desulfurization and nitrification efficiencies were maintained at 100% under 100-mg-S L^{-1} d^{-1} of SLR and 283±27-mg-N L^{-1} d^{-1} of NLR as same with the experiment in chapter 3 (data was not shown). The amount of produced SO_4^{2-} was almost the same as the amount of supplied H_2S into the reactor at 0.5%, indicating that supplied H_2S was completely oxidized to SO_4^{2-} in the CSTMR. O_2 contaminated into desulfurized biogas was 0.36±0.06% on average which is lower than the upper limit for natural gas grids (typically <0.5%, Munoz et al., 2015).

The biomass concentration of *C. sorokiniana* NIES-2173 in the airlift reactor was maintained in the range of 0.47–1.05 g-dry weight (DW) L^{-1} from day 3 to day 11 under the semi-continuous operation (Figure 5-3, (A)). The growth rate tended to decrease after day 12, however it started to increase again by washing of the inside reactor on day 14. Attachment of microalgae to the surface of the reactor probably reduced the light permeability of the reactor. To remove the effect of over-shading by microalgal attachment on the reactor surface, the data from day 7 to day 11 were used for process evaluation as a steady period. During this period, the volume and area productivity of *C. sorokiniana* were 0.48±0.03 g-DW L^{-1} d^{-1} and 9.8±0.5 g m^{-2} d^{-1}, respectively (Figure 5-3, (B)). Because this value is in the range of commercial-scale microalgal production, 3.3–20 g-DW m^{-2} d^{-1} of *Chlorella* and *Spirulina* (Melis, 2009), the obtained productivity is reasonable as an example of the coupling process operation.

During the steady period of the microalgal reactor (days 7–11), the average of the NH_4^+ concentration of the ADE, NO_3^- concentration of SDN effluent, and effluent of microalgal cultivation

were 833.8±7.6 mg-N L^{-1}, 827.2±34.9 mg-N $L^{-1,}$ and 639.5±18.3 mg-N L^{-1}, respectively (Figure 5-4). The reduction of NO_3^- through microalgal cultivation with 5-day HRT was 187.7 mg-N L^{-1} (23% reduction and 37.5-mg-N L^{-1} d^{-1} removal rate).

5.2.2. Gases consumed and produced in the SDN and microalgal cultivation

At the end of the experiment, the changes in the volume and O_2 concentration of a gas bag that stores O_2 were measured every 24 hours to reveal the O_2 amount consumed in the SDN treatment. The amount of O_2 consumed in SDN was 4.1±0.1 NL $reactor^{-1}$ d^{-1} on average (1.36 NL L^{-1} d^{-1}, 60.6 mmol L^{-1} d^{-1}) (Figure 5-5) which was 1.5 times higher than the total amount of O_2 removed by contamination into desulfurized biogas, stoichiometric amounts of O_2 consumed for nitrification and desulfurization calculated from NH_4^+ and H_2S loading rates. Other reactions consuming O_2 such as respiration of bacteria, decomposition of organic compounds derived from ADE, and dead cells probably proceeded in the SDN.

Figure 5-6 shows the volumes of consumed CO_2 and recovered O_2 in the microalgal cultivation calculated by volume and compositional data of gasbags. Both values fluctuated in the range of 1–5 L except O_2 produced on day 1. The averages in day 7–11 were 2.9±1.0 NL-CO_2 $reactor^{-1}$ d^{-1} and 3.4±0.7 NL-O_2 $reactor^{-1}$ d^{-1}, respectively. The ratio of recovered O_2 to consumed CO_2 was 1.2. When biomass composition follows the Redfield ratio which is $CH_2O(NH_3)_{0.15}$ (Redfield, 1963), and NO_3^- is the nitrogen source, the ratio can be expected as 1.3 (Eriksen et al., 2007). The ratio was also measured experimentally under using NO_3^- and reported as 1.1–1.2 of *Phaeodactylum tricornutum* (Sobczuk et al., 2000) and 1.0–2.0 of *Chlorella* sp. (Eriksen et al., 2007).

5.2.3. Mass balance in the coupling process

The measured mass flow of the operated experimental process was summarized in Figure 5-7. Supplied synthetic biogas without CO_2 and 0.45-μm filtered ADE were completely desulfurized/nitrified simultaneously in the CSTMR with 100 mg-S L^{-1} d^{-1} of SLR and 278 mg-N L^{-1} d^{-1} of NLR. O_2 contamination into desulfurized biogas was suppressed well until 0.4% by using an external O_2 bubbling column. Microalga was cultivated with high enough biomass productivity (0.48

g-DW L^{-1} d^{-1}) and 30 mmol L^{-1} d^{-1} of O_2 production using undiluted SDN effluent. Based on the obtained mass balance, the process operation condition such as reactor volume, HRT, and loading rate can be designed depending on the substrate concentrations and required treatment efficiencies. For example, based on the NO_3^- removal rate in the microalgal cultivation, it was considered that four times longer HRT (20-day HRT) is required under the same reactor operation conditions with this experiment for the removal of NO_3^- from supplied SDN effluent until less than 100 mg-N L^{-1} which is the national minimum effluent standards in Japan, i.e., a volume ratio of 3 to 20 may appropriate for SDN and microalgal cultivation reactors. While, when microalgae are cultivated outside, the HRT of photobioreactor will be approximately 40 days based on the assumption of 12 hour:12 hour light: dark cycle and 600 μmol-photons m^{-2} s^{-1} of light intensity which is the same with this experimental condition. Note that, O_2 production in the microalgal reactor can be assumed to be larger than O_2 consumed in the SDN reactor under both light conditions.

5.3. Assessment and future challenges of the coupling process of SDN and microalgal cultivation

Here, the recovery method of pure O_2 produced by microalgae from mixed gas was further considered. Then, economic and electrical energy consumption were assessed about the developed process, and these were compared with conventional processes. Last, the robustness of the developed process was discussed.

5.3.1. Future challenges—O_2 recovery with high concentration from microalgae culture

In the present study, the following challenges remain: (1) development of technology such as membrane separation for CO_2 removal from biogas before SDN treatment, (2) high salinity of SDN effluent, which requires consideration of the microalgae strain used in the process, (3) construction and operation of a relatively complicated SDN reactor which is CSTMR with an external membrane module and a pure O_2 bubbling column, might prevent implementation of the developed process to the waste treatment facilities. Additionally, to utilize produced O_2 in the SDN with high concentration,

which enables to reduce electrical energy consumption for the mechanical aeration and to avoid N_2 contamination into biogas, a recovery method of pure O_2 from microalgae culture is desired.

In microalgal cultivation, enough aeration of the culture with/without CO_2 is often required for the CO_2 supply and removal of accumulated O_2 which inhibits microalgal growth (Fuentes et al., 1999), from the culture. Because the aeration consumes a large amount of electrical energy, membrane technologies have been developed such as hollow-fiber membrane apparatus and membrane photobioreactor (Cogne et al., 2005; Kishi et al., 2020), other than attempts to improve gas-liquid contacting efficiency by using horizontal two-phase flow photobioreactor (Rehl and Müller, 2011), and internal mixers (Ugwu et al., 2002). Among them, the hollow-fiber membrane may have the potential to adapt to our challenge: recovery of high concentration of O_2 from the culture. Usually, CO_2 supply and O_2 removal in the culture are achieved by CO_2 supply to the outer side of the membrane in the conventional hollow-fiber membrane module. This causes the undesired CO_2 contamination into recovered O_2. Therefore, for the O_2 recovery in the developed process, it may be better to evacuate the outer side of the membrane by a vacuum pump instead of a CO_2 supply. However, this operation may easily cause algae clogging of the membrane, so further studies are necessary about the pore size of the membrane, pump pressure, and maintenance method of the membrane, etc.

Another way of the O_2 recovery from photobioreactor is by CO_2 supply into the reactor. The pH of the microalgae culture tends to increase mainly by DIC assimilation of microalgae. Therefore, CO_2 supply is required for the pH adjustment and compensation of DIC. When the high concentration of CO_2 is bubbled in the culture, CO_2 is gradually dissolved into the culture, and dissolved O_2 is transferred into the bubbles. That is, the O_2 partial pressure of the bubbles increases with the decrease in CO_2 partial pressure. It is desired basically to avoid CO_2 contamination into recovered O_2 as mentioned above. However, even if 50% of CO_2 is contaminated into O_2 gas and supplied into SDN reactor with O_2, the amount of contaminated CO_2 is less than 10% of CH_4 supplied into SDN reactor based on the mass balance data obtained in chapter 5. Therefore, the inhibition effect of contaminated

CO_2 on nitrification is probably negligibly small. Furthermore, contaminated CO_2 in desulfurized gas will be removed by the 2nd membrane gas separation module after SDN. Therefore, it is also considered that the CO_2 contaminated into O_2 does not affect the CH_4 purification efficiency. Then, a sparge of pure CO_2 derived from biogas into the microalgae culture may be a possible way of O_2 recovery. However, an excessive supply of pure CO_2 causes a significant drop in pH and leads to the collapse of the microalgal reactor. As far as I know, the previous study which cultivated microalgae using pure CO_2 without air is none. It is necessary to consider an appropriate method to recover O_2 effectively by pure CO_2 as less CO_2 dissolution as possible.

5.3.2. Economic and electrical energy consumption estimation

To evaluate the process applicability, the economic of the developed process were briefly estimated, and the results were compared with some conventional biogas and ADE treatment processes. Also, electrical energy consumption was estimated as an example of the environmental loads of the developed process. Figure 5-8 shows the mass balance of the process treating 14000 Nm^3 biogas (0.3% H_2S) and 200 m^3 ADE (800 mg-N L^{-1}) simultaneously. HRT of SDN was 3 days. Microalgae are cultivated in outdoor spaces and 12 hours:12 hours of light:dark periods and 600 μmol-photons m^{-2} s^{-1} of light intensity were estimated, therefore, HRT was set to 40 days to reduce nitrogen concentration of effluent. Because of a lack of conventional technology, it was assumed that O_2 produced by microalgae is released into the atmosphere without utilization, and pure O_2 generated by the PSA method was supplied into the SDN reactor. As a result of CO_2 removal from biogas, SDN and microalgal cultivation, 8400 m^3 of biomethane (more than 98% of CH_4 without H_2S), 200 m^3 of effluent with 40 g-N m^{-3} of dissolved inorganic nitrogen, 1920 kg-DW of microalgae biomass are produced in a day. Figure 5-9 shows the schematic diagram of the process. In this evaluation, the process was separated into four units: (a) two membrane modules and a compressor for CO_2 removal from biogas; (b) a PSA O_2 generator; (c) a CSTMR consisted of a three-stage CSTR (two are for O_2 aeration and one is for biogas supply), a side-stream membrane sludge separator (external membrane module), and pumps for wastewater circulation and effluent discharge; and (d) horizontal tubular

photobioreactor (16 ha) and other equipment related with microalgal cultivation. After that, the capital cost, annual cost consisted of running cost and depreciation expenses, and the amount of electrical energy consumption was estimated about each unit, respectively, using the data of previous studies that evaluated a similar treatment process. The summary of the calculation and references were shown in Table 5-2. For the cost comparison, two processes were evaluated as conventional treatments of biogas and ADE. One process consisted of aerobic biodesulfurization (Cano et al., 2018), combined heat and power (CHP) system (Caposciutti et al., 2020), and nitrogen removal method of Anaerobic–Anoxic–Oxic (A_2O) (Harris et al., 1982). Another process used chemical scrubbing (Cano et al., 2018) instead of biodesulfurization for biogas. These processes were estimated based on each reference. Cost and energy consumption in the anaerobic digestion reactor (around 8000 m^3 of industrial-scale) which is the producer of biogas and ADE was not included in all estimation.

Table 5-9 summarized estimated capital cost, annual cost, and annual electric energy consumption of the proposed process. Microalgal cultivation with a largest reactor scale showed higher capital cost, running cost, annual electricity consumption, and also revenue than SDN treatment. CO_2 removal and O_2 production took large amounts of costs and electricity consumption in the SDN treatment facilities. Capital cost, annual running cost, and electricity consumption of these treatments accounted for 91%, 56% and 94% of the total value of the SDN, respectively. Note that polyimide material having relatively lower H_2S/CH_4 and higher CO_2/CH_4 selectivity among commercialized membranes was assumed to be used with 100 \$ m^{-2} of price for CO_2 separation from biogas. In this proposed process, the revenue from biomethane and microalgae biomass sales exceeded expenditure in both SDN and microalgal cultivation, and then, the negative net annual cost was obtained (total 5.21 M\$ y^{-1} of profit). Both selling prices were assumed without shipping expenses. Assumed selling price of biomethane (0.8\$ Nm^{-3}) (Caposciutti et al., 2020) is higher than natural gas (0.24 \$ y^{-1}) considering subsides; therefore, the selling price varies by country. The price of microalgal biomass was assumed as 10 \$ DW-kg^{-1} for food; however, this price is different depending on the end product.

The capital and annual cost of the proposed coupling process were compared with conventional processes in Figure 5-10. The capital cost of the coupling process was 10- and 11-times higher than conventional processes (1) and (2), respectively (Figure 5-10-(A)). On the other hand, the difference of expenditure between the coupling process and conventional processes was reduced in the annual cost (Figure 5-10-(B)) because of reduction of mechanical aeration for ADE nitrification, methanol addition for denitrification, and biogas desulfurization system. The estimated revenue of the coupling process was 6.5-times higher than conventional processes, resulting in that more than 12-times higher net cost was obtained in the coupling process compared with that in the conventional processes.

Market prices of low safety requirement products of microalgae such as biofuels, fertilizer, bioplastics are reported as less than 1 $ DW-kg^{-1} (Acién Fernández et al., 2019). However, based on the estimated 3.23 M$ y^{-1} of expenditure and 701 ton-DW y^{-1} of biomass production in the microalgal cultivation, more than 4.6 $ kg-DW^{-1} of the selling price is required to implement the proposed process. Pure cultivation of microalgae in the proposed process has the potential to cultivate microalgae for high-value end products such as food and cosmetics due to avoidance of bacterial contamination. It was also revealed in the present thesis that most types of toxic heavy metals of ADE can be removed in the SDN treatment (section 4.3.1). However, the establishment of a safety standard and management method is still required to sell obtained biomass for high-value end products. Using crop residues and/or food wastes as AD substrate rather than municipal solid waste or sewage sludge is also an effective way to manage ADE composition. On the other hand, even if the quality of obtained biomass cannot reach the required level for human food due to waste usage, prices for feed additives for aquaculture and livestock are also high, from several dozen to several thousand dollars per kg-DW (Acién Fernández et al., 2019). Some microalgal species such as *Chlorella zofingiensis* which is the same genus of *C. sorokiniana* used in the present study and *Hematococcus* can accumulate beneficial ingredients such as unsaturated fatty acid and antioxidants under stress condition (e.g., nitrogen limitation, high salinity, strong light intensity) (Boussiba and Vonshak, 1991;

Bar et al., 1995). Therefore, beneficial ingredients may be obtained by temporal exposure of microalgae biomass cultivated in the coupling process to stress condition, and extraction process, and sold as feed additives. For the accurate estimation of economic and environmental loads, pilot- and full-scale operation of the developed process under introducing equipment such as the CO_2 separation membrane module and oxygen generator is necessary.

5.3.3. Requirements for the process installation

Requirements for scaled-up reactors and installation area should be arranged to install the proposed coupling process. In the scale-up of SDN treatment, biogas-liquid contact efficiency in the CSTMR should be adjusted to be low enough to avoid O_2 contamination from liquid to desulfurized biogas. Equilibrium O_2 concentration in gas is around 8% to 3.0 mg-O_2 L^{-1} of DO concentration in water at 30ºC based on 4.75×10^4 atom (molar fraction)$^{-1}$ of Henry's law constant (Wilhelm et al., 1977). To reduce O_2 contamination into biogas until less than 0.5%, the gas-liquid contact efficiency should be taken attention to be low to prevent O_2 contamination to desulfurized biogas, rather than to reach high to dissolve H_2S well since the solubility of H_2S in water is very high compared with O_2 (0.0609×10^4 atom (molar fraction)$^{-1}$ of Henry's law constant; Wilhelm et al., 1977). In microalgal cultivation, it is probably preferred to use a tubular photobioreactor to recover O_2 from the culture because installing a few degassing devices in a long tube may allow to effective recovery of dissolved O_2 from the culture with a high concentration.

The installation area of the proposed process may be mainly limited by the suitability of environmental conditions for microalgal cultivation, the land availability, and ease of access to AD substrate and sales channel of biomethane and microalgae biomass in addition to enough technical support system for process operation and maintenance. For the outdoor mass cultivation of microalgae, enough light intensity, day length and temperature are necessary. Therefore, commercial application has been expanded especially in countries located at low latitudes such as Hawaii, New Mexico, Israel, California, New Zealand, Philippine (Park et al, 2011). The high biomass yield of microalgae has also been estimated in such countries as Australia, Brazil, Colombia, Egypt, Ethiopia,

India, Kenya, and Saudi Arabia (Moody et al., 2014). However, even in Japan, there is a possibility that microalgae can be cultivated in Shikoku and Kyushu areas in addition to Okinawa, which are relatively warm even in winter. Besides, the increase in net cost with the decrease in process scale can be expected in both the SDN and microalgal cultivation systems. Therefore, the high land availability and ease of access to AD substrate are necessary to operate the process with enough scale. Also, to sell biomethane and microalgal biomass at a high price, using food waste and crop residue as AD substrate rather than animal manure and mixed organic waste, and obtaining local sales channels of biomethane and microalgae biomass.

5.3.4. The robustness of the coupling process of SDN and microalgal cultivation

Regarding SDN, although 100 mg-S L^{-1} d^{-1} of SLR demonstrated in the present thesis was expected to be almost the upper value in simultaneous biogas and ADE treatment (section 2.3.4., chapter 2), efficiencies of desulfurization and nitrification did not decrease for even a day while changing the SLR in the experiment. This SLR value is lower than conventional biological desulfurization treatments (950–4400 mg-S L^{-1} d^{-1}; Muñoz et al., 2015). Therefore, the SDN treatment efficiencies may have relatively high robustness against changes in SLR. However, since the acclimatization of microbes to sulfide needs 1–2 months, recovery takes a long time when the reactor collapses by the operational troubles or significant compositional change of substrates. Equipment, especially which have membranes, should be carefully managed. Separation of the process to a few units is also a way to avoid troubles. The preparation of a chemical desulfurization system is also possible for when SDN treatment collapses because the installation cost of the chemical desulfurization system is relatively low in contrast to high running cost (Cano et al., 2018).

In the microalgal cultivation, since nitrogen is supplied as NO_3^- instead of NH_4^+, NH_3 inhibition does not cause even when the pH of the culture cannot be controlled completely, indicating higher robustness than the conventional microalgal cultivation using ADE. However, it is difficult to maintain high NO_3^- removal efficiency compared with the traditional denitrification process due to solar radiation and day length fluctuations. It is better to connect drainpipes to the sewage treatment

plant or a lagoon for a post-treatment instead of directly discharging effluent into the natural water bodies.

Table 5-1. Supplementary information about experimental conditions in the coupling operation of simultaneous desulfurization-nitrification (SDN) and microalgal cultivation and mass balance estimation.

Experimental details
SDN treatment of synthetic biogas without CO_2 and anaerobic digestion effluent (ADE)
Reactor set up
• A continuous stirred tank membrane reactor (CSTMR) used in chapter 3 was operated continuously with acclimatized microbes
• Effective volume: 3.0 L
Operation conditions
• Same with Phase 4 in chapter 3
• Hydraulic retention time (HRT): 3 days
• Temperature: 30°C; pH: 7.5
Microalgal cultivation using ADE treated by SDN
Reactor set up
• 5.0-L acrylic airlift photo-bioreactor was used with acclimatized microbes
• Diameters of outer and inner tubes were 10 cm and 7.4 cm, respectively
• Effective volume: 4.5 L
Operation conditions
• SDN effluent was supplied after the addition of $MgSO_4$ and trace metals with 5 times higher concentration of C-medium
• HRT: 3 days; Sludge retention time (SRT): 2 days
• Air stocked in a gas bag was continuously supplied through an ethylene-propylene rubber with 1 mm slits attached to the bottom of the reactor with about 1.0 L min^{-1} flow rate.
• Sampling: 2.25 L of culture was discharged every day, and a part of the culture was centrifuged at 4000 × g for 15 min. Obtained 1.35 L of supernatant was re-supplied into the reactor with 0.9 mL of new SDN effluent through 0.22-μm pore autoclaved PES filter (GPWP0477; Merck Millipore, USA)
• Temperature: 30°C; pH: 7.5; light intensity: 600 μmol-photons m^{-2} s^{-1} (continuous)

Table 5-2. Database and assumptions for the calculation of cost and consumed electrical energy of the developed process.

Unit process	Data source/assumption
(a) Membrane modules and a compressor for CO_2 removal from biogas	• Cost was estimated based on Chu and He (2018) • Membrane: Polyimide (price: 100 \$ m^{-2})
(b) A PSA O_2 generator	• Cost was estimated based on "Pure oxygen activated sludge" in Harris et al. (1982) [a]
(c)-1 A CSTR separated to three stages	• Cost was estimated based on "Pure oxygen activated sludge" in Harris et al. (1982) [a] • One stage is for biogas supply and others were for O_2 supply • Surface aerator is used for O_2 supply and agitation • Blower energy with combined blower and motor efficiency of 0.61: 0.049 kWh m^{-3}-gas (Leu et al., 2009) • $Ca(OH)_3$ was consumed for pH adjustment
(c)-2 A side-stream membrane sludge separator (external membrane module) and sludge circulation pump	• Cost of membrane separator was estimated based on Judd (2016) • Membrane: 87\$ m^{-2} was assumed [a] • 50% citric acid and 14% sodium hypochlorite were used for membrane cleaning (2 gal m^{-3}-influent) [a] • Sludge (with wastewater) circulation rate was 1530 m^3 d^{-1} which also helps agitation of CSTR • Cost of pumps for sludge circulation and effluent discharge was estimated based on "Pumping" in Harris et al. (1982)[a]
(d) Horizontal tubular photobioreactor (16 ha) and other equipment related with microalgae cultivation	• The required area (16 ha) was calculated based on the microalgae productivity per volume and nutrient removal rate obtained in the experiment • Cost of photobioreactor was estimated based on Norsker et al. (2011). Seawater pump station and chemicals for medium was excluded from the calculation • Day length was 12 hour with 600 µmol-photons m^{-2} s^{-1} on average
Others	• Correction of equipment cost: Reported equipment prices were corrected to the price in 2018 by using Marshall and Swift Index, Engineering News Records Cost Index, Pipe Cost Index and Chemical Plant Cost Index • Equipment life: Structures: 30 y; Process equipment such as pump, O_2 generator: 15 y; Consumable equipment (CO_2/sludge separation membrane): 8 y; tubes in photoreactor: 1 y • Labor rete: 51.5 \$ man-$hour^{-1}$; Electricity: 0.1 \$ kWh^{-1}: Land: 20000 \$ $acre^{-1}$ • Revenue : Biomethane: 0.8 \$ Nm^{-3}; Electricity: 0.11 \$ kWh^{-1}; heat \$/kWh; Heat usage on site: 0.024 \$ kWh^{-1} (instead of natural gas usage); Microalgae biomass: 10 \$ kg-DW^{-1}

a: Equipment cost database in CAPDET Works 4.0 software (2018) by Hydromantis Environmental Software Solutions, Inc. was used for calculation

Table 5-3. Estimated capital cost, annual cost and annual electricity consumption of the coupling process of SDN and microalgae cultivation in a practical application scale (daily treatment of 14000-m^3 biogas and 200-m^3 anaerobic digestion effluent).

	SDN treatment				Microalgal cultivation (Photobioreactor and others)	Total
	CO_2 removal from biogas using membrane	O_2 production using PSA system	CSTMR	Total		
Capital cost (M$)	**3.61**	**1.63**	**0.49**	**5.73**	**10.34**	**16.07**
Net annual cost (M$ y^{-1})	—	—	—	**-1.43**	**-3.78**	**-5.21**
Installation cost* (M$ y^{-1})	0.32	0.11	0.02	0.54	0.72	1.17
Running cost (M$ y^{-1})	0.16	0.07	0.18	0.41	2.08	2.50
Labor cost (M$ y^{-1})	—	—	—	0.16	0.43	0.59
Revenue (M$ y^{-1})	—	—	—	2.45	7.01	9.46
Annual electricity consumption (GW y^{-1})	**1.34**	**0.54**	**0.11**	**1.99**	**15.64**	**17.63**

*Installation cost was divided by life time of each equipment.

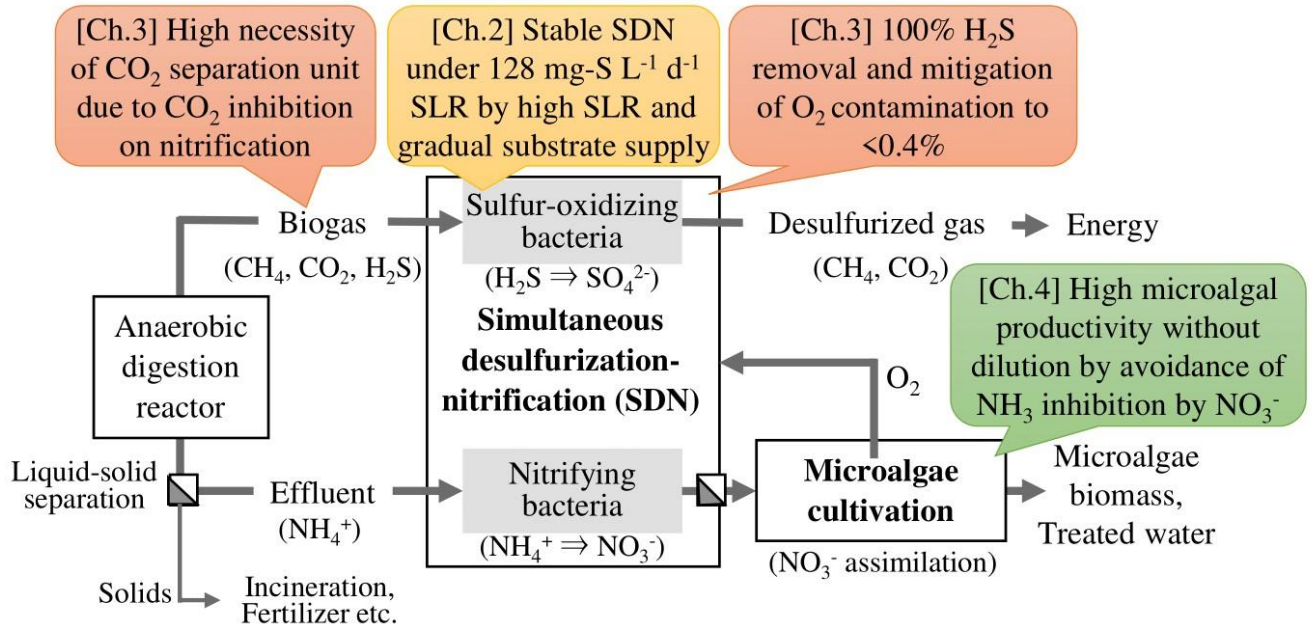


Figure 5-1. Main findings/achievements for the process development in each chapter.

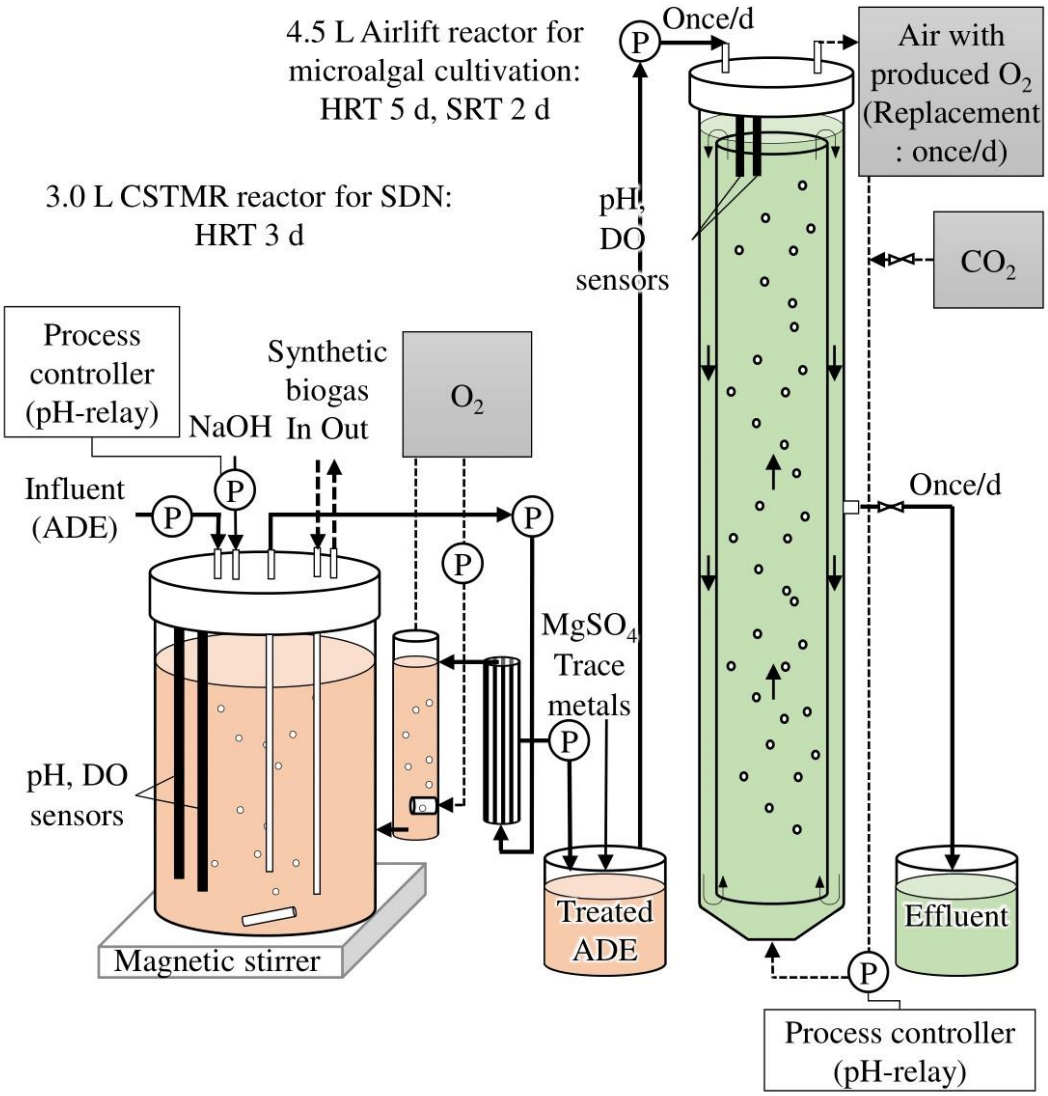


Figure 5-2. Schematic diagram of a continuous stirred tank membrane reactor (CSTMR) for simultaneous desulfurization-nitrification (SDN) and airlift reactor for microalgal cultivation.

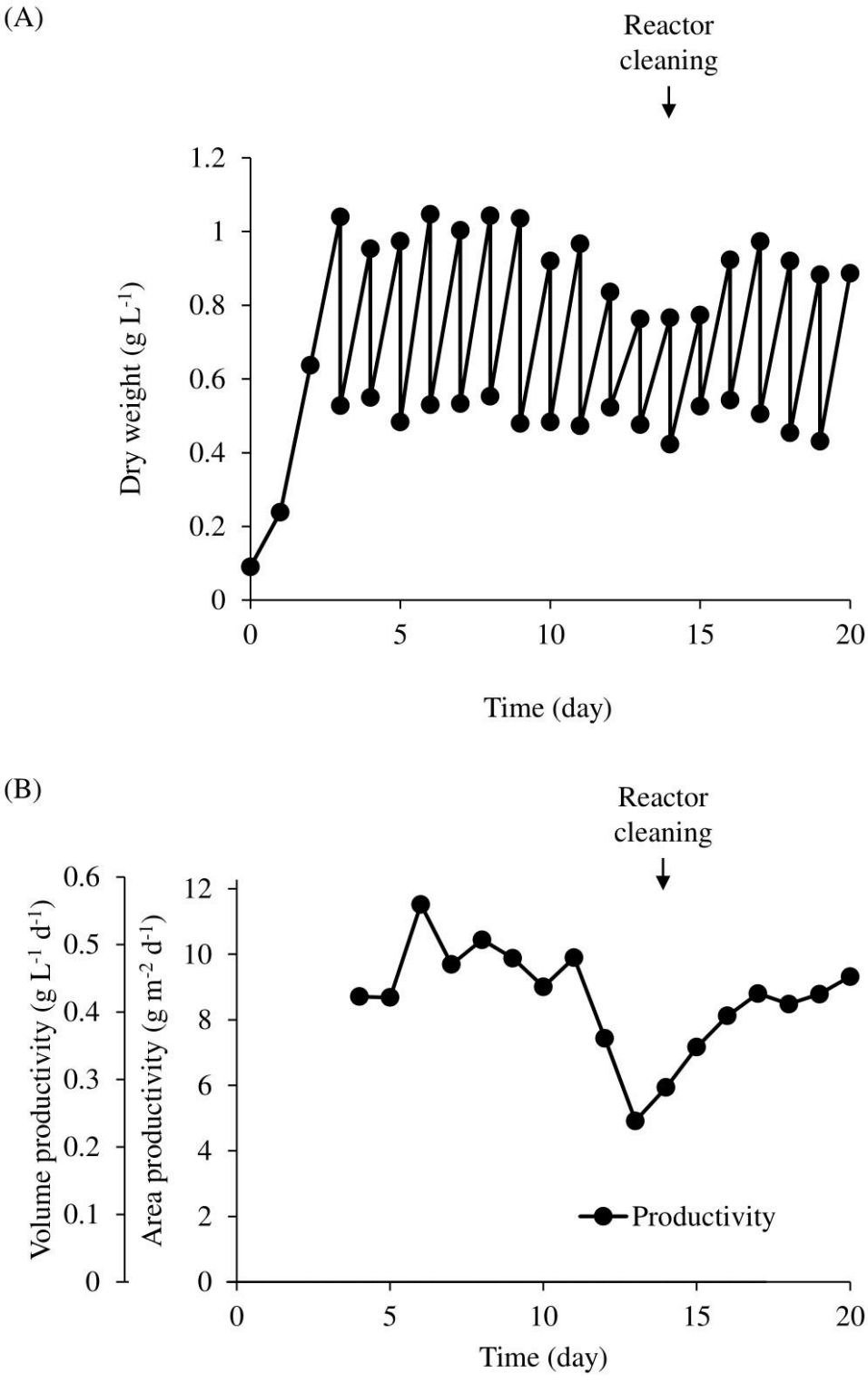


Figure 5-3. Time course of dry weight (A), volume and area productivity (B) of *C. sorokiniana* in the microalgal cultivation reactor.

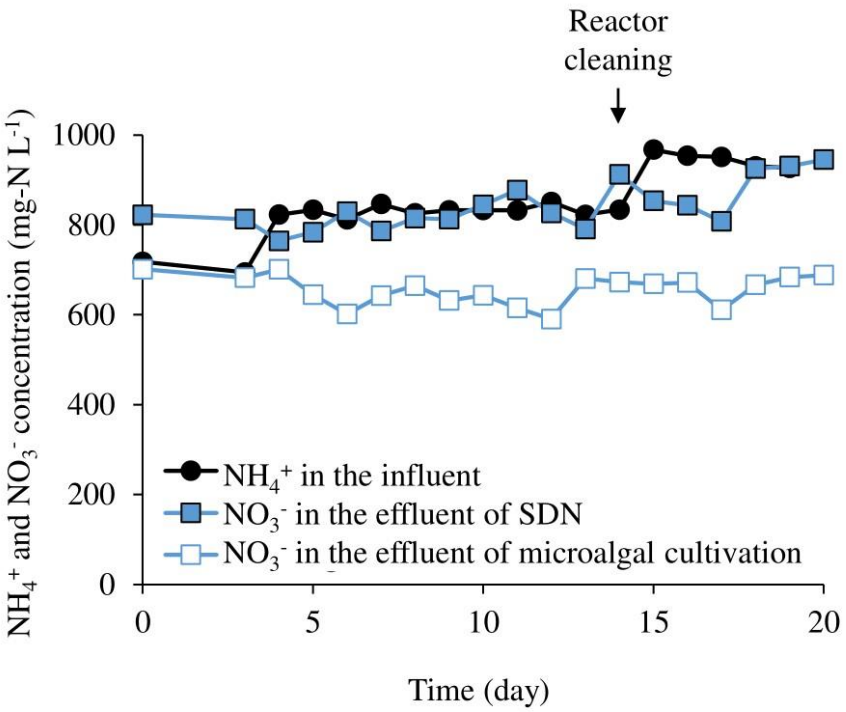


Figure 5-4. Time course of NH_4^+ concentration in the influent, NO_3^- concentration in the effluents of simultaneous desulfurization-nitrification (SDN) and microalgal cultivation reactors.

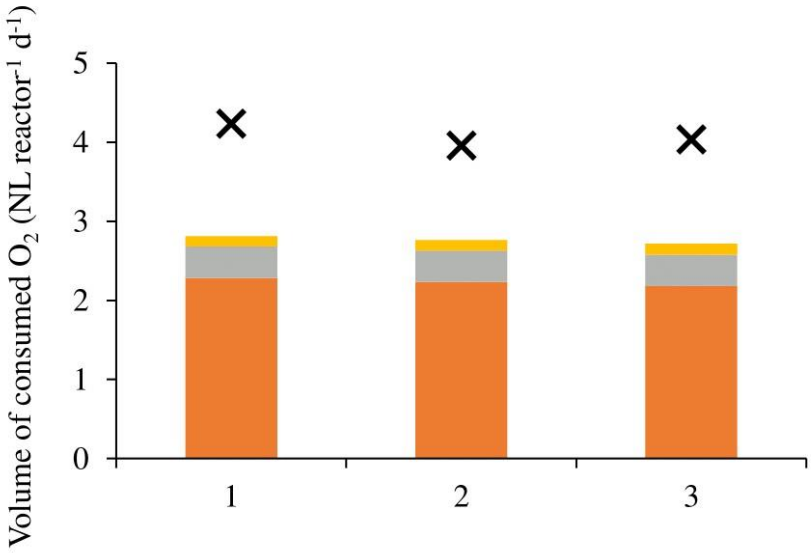


Figure 5-5. Comparison between the actual amount of O_2 consumed in the simultaneous desulfurization-nitrification (SDN) (×) and O_2 contaminated into biogas (■) plus stoichiometric amounts of O_2 consumed for nitrification (■) and desulfurization (■) calculated from each loading rates.

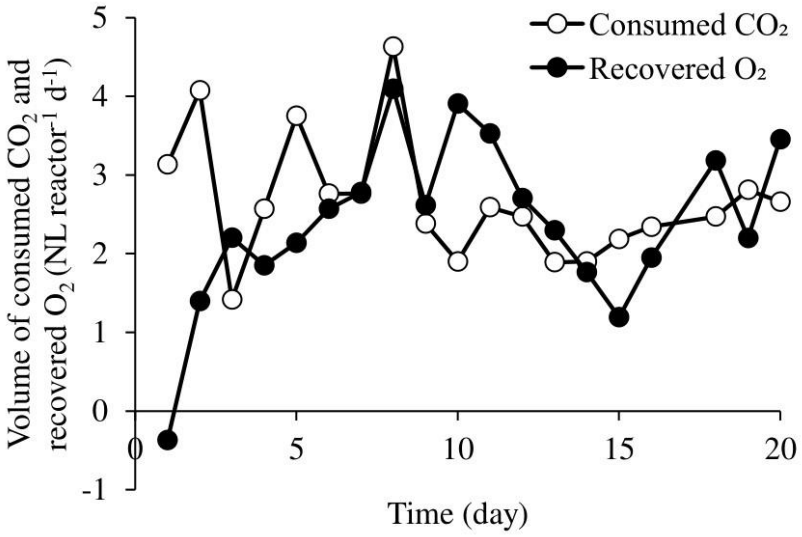


Figure 5-6. Time course of volumes of consumed CO_2 and recovered O_2 in the microalgal cultivation.

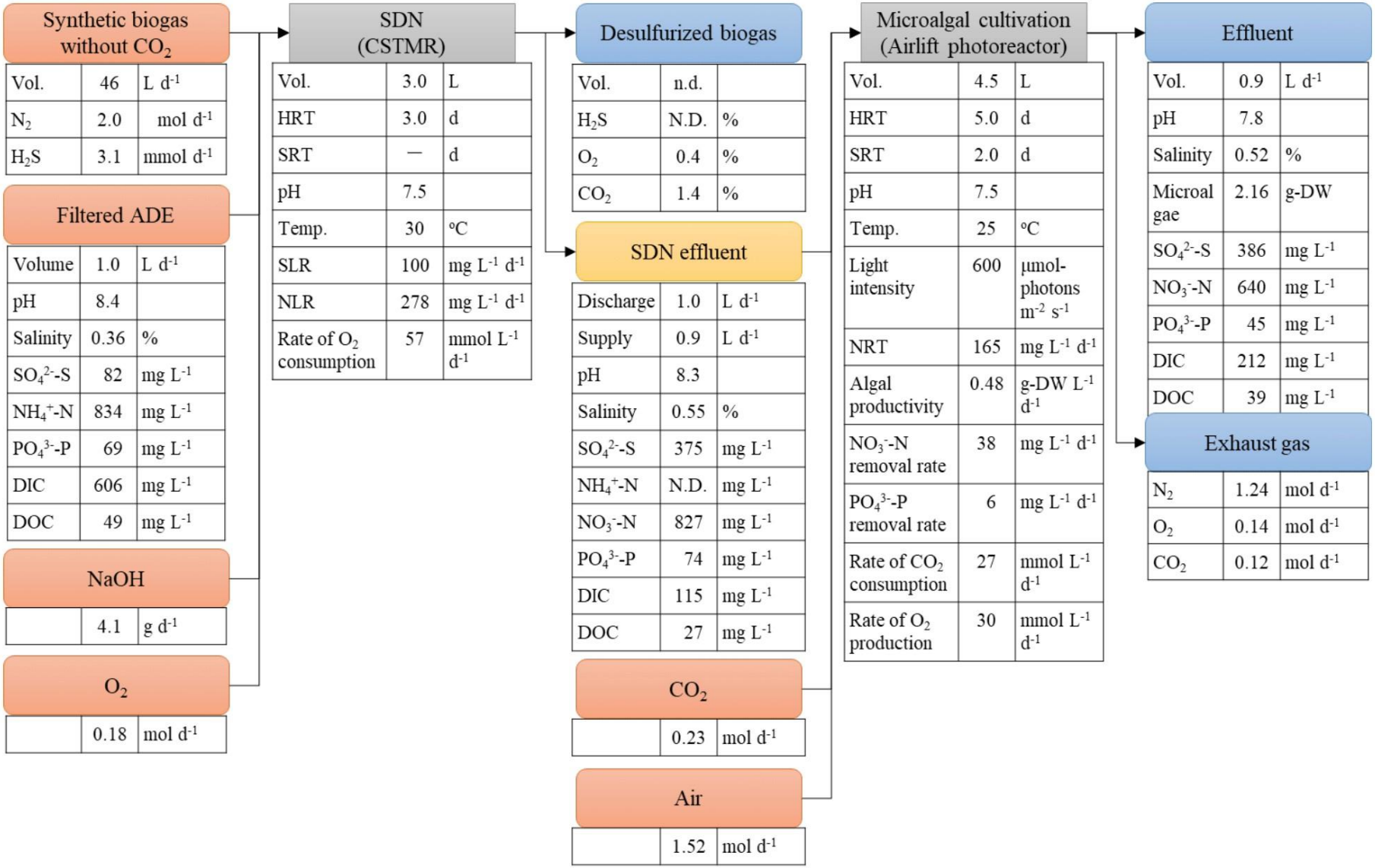


Figure 5-7. Mass flow of coupling process of SDN and microalgal cultivation operated in the experiment. Red box: input; yellow box: intermediate; blue box: output; gay box: process. Blue solid: gas; black solid: liquid. HRT: hydraulic retention time; SRT: sulfide loading rate; NRT: nitrogen loading rate. n.d.: not determined; N.D.: not detected.

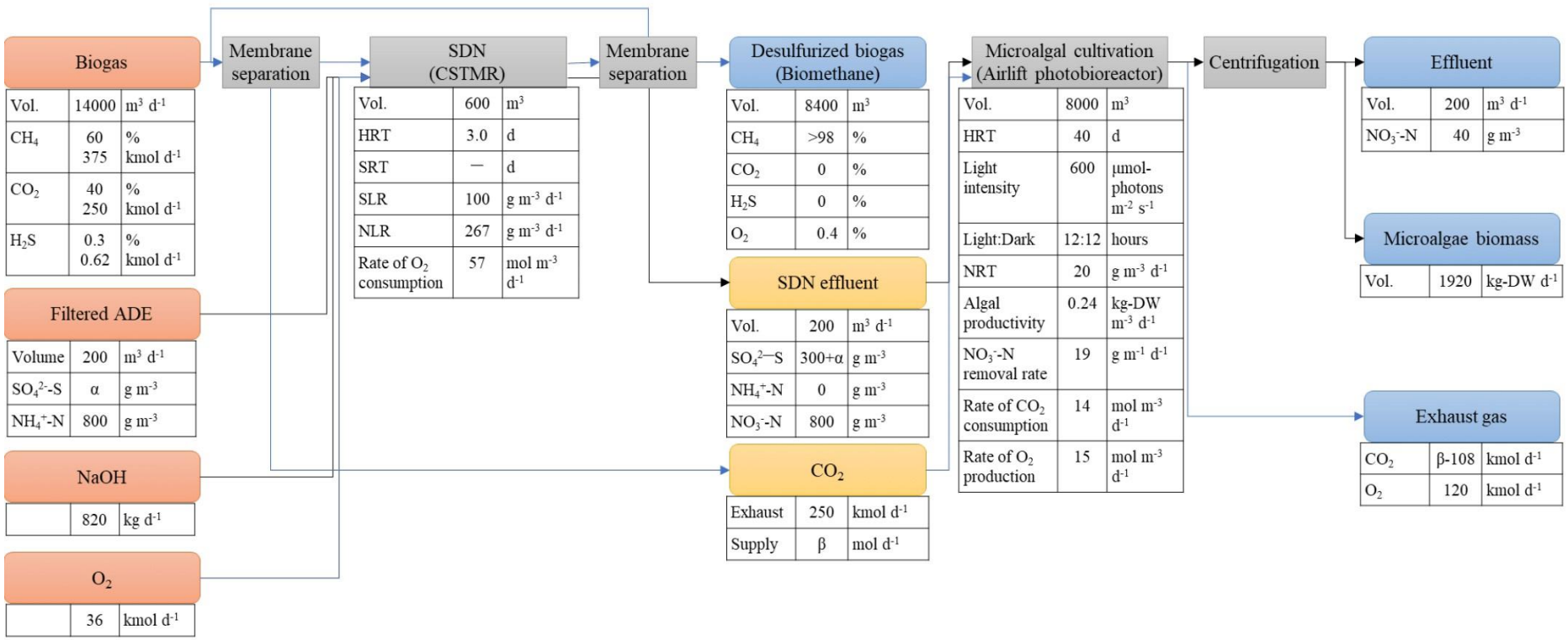


Figure 5-8. Assumed mass balance of the coupling process of SDN and microalgae cultivation in a practical application scale. Red box: input; yellow box: intermediate; blue box: output; gay box: process. Blue solid: gas; black solid: liquid. HRT: hydraulic retention time; SRT: sulfide loading rate; NRT: nitrogen loading rate.

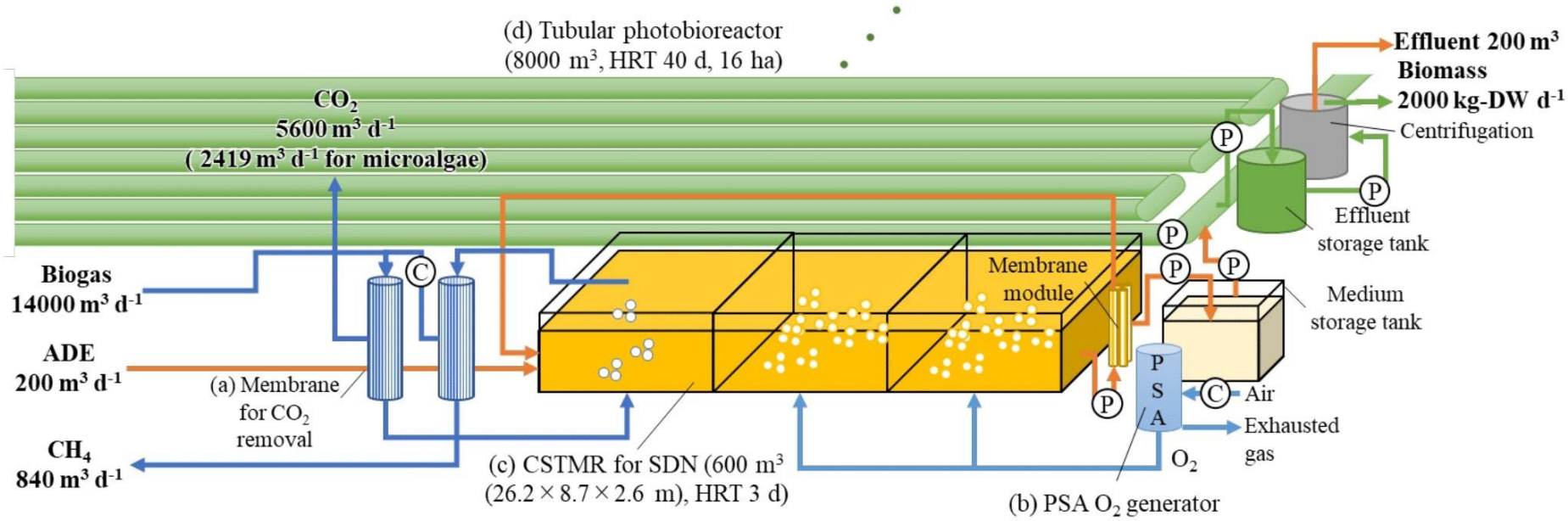


Figure 5-9. Schematic diagram of a practical application form of biogas and ADE treatment by coupling process of SDN and microalgae cultivation. Blue lines are gas phase, and orange and green lines are liquid phase. P: pump; C: compressor.

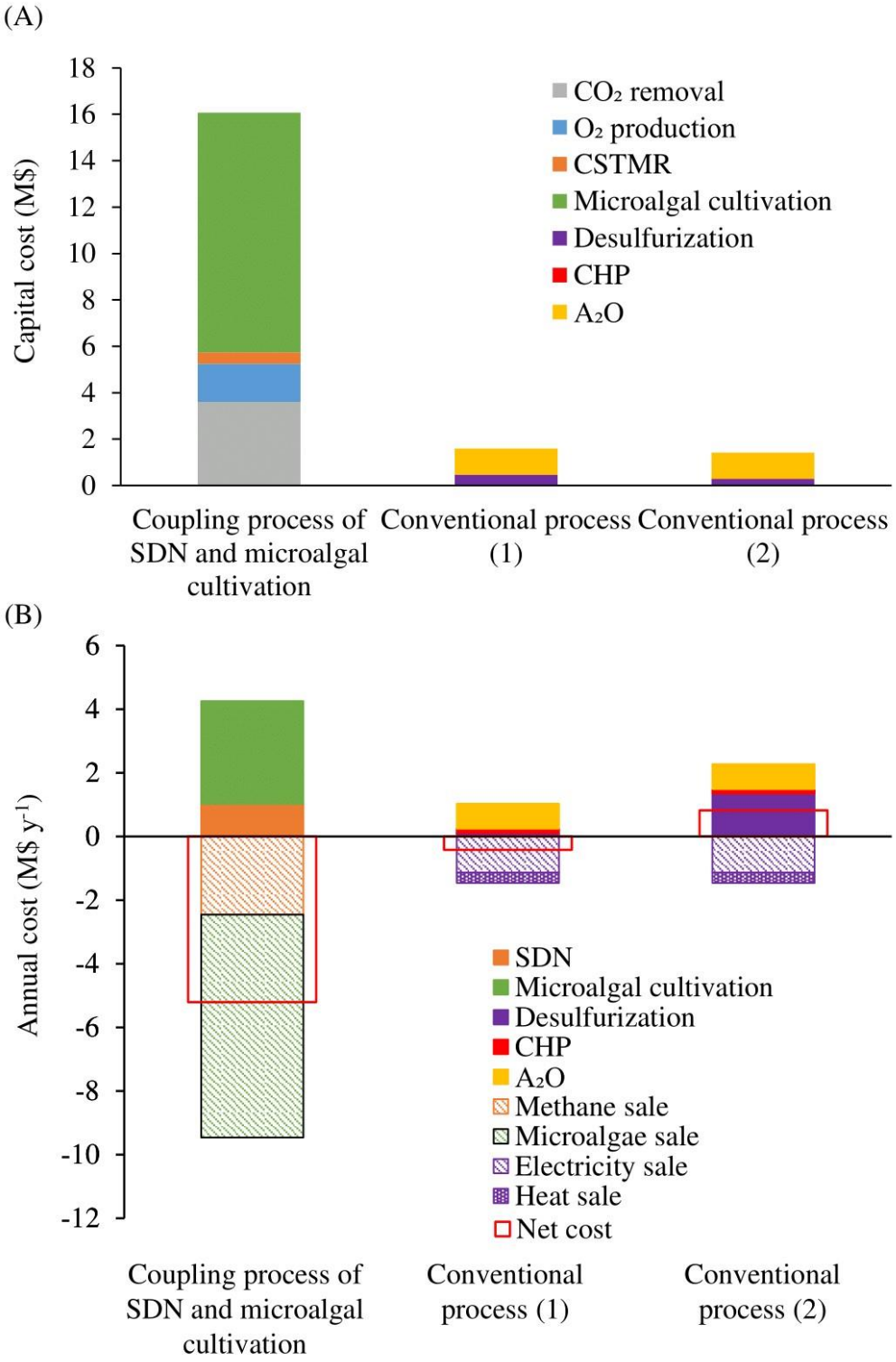


Figure 5-10. The capital cost (A) and annual cost (B) of facilities for biogas and ADE treatment: the coupling process of SDN and microalgae cultivation and conventional processes using bio-desulfurization (1) or chemical desulfurization (2).

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