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Effect of Algal Inoculation on COD and Nitrogen Removal, and Indigenous Bacterial Dynamics in Municipal Wastewater

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The effects of algal inoculation on chemical oxygen demand (COD) and total nitrogen (TN) removal, and indigenous bacterial dynamics were investigated in municipal wastewater. Experiments were conducted with municipal wastewater inoculated with either *Chlorella vulgaris* AG10032, *Selenastrum gracile* UTEX 325, or *Scenedesmus quadricauda* AG 10308. *C. vulgaris* and *S. gracile* as fast growing algae in municipal wastewater, performed high COD and TN removal in contrast to *Sc. quadricauda*. The indigenous bacterial dynamics revealed by 16S rRNA gene amplification showed different bacterial shifts in response to different algal inoculations. The dominant bacterial genera of either algal case were characterized as heterotrophic nitrifying bacteria. Our results suggest that selection of indigenous bacteria that symbiotically interact with algal species is important for better performance of wastewater treatment.

Keywords: Municipal wastewater, algal inoculation, bacterial dynamics, COD and nitrogen removal, algal-bacterial consortia

Introduction

Nutrients and organic carbon in wastewater discharge have negative impacts on nature and the quality of drinking water. Excessive nutrients (*i.e.*, nitrogen and phosphorus) can cause eutrophication, which is a serious environmental concern, because it reduces biodiversity, upsets the balance of the microbial community, causes odor, and increases water toxicity [2]. Organic carbon in wastewater discharge can also be problematic, since microorganisms that utilize organic carbon as energy substrates simultaneously consume dissolved oxygen in water bodies and become a threat to aquatic life [29]. Therefore, removal of nutrients and organic carbon to acceptable levels is a major requirement in wastewater treatment [2].

Current wastewater treatment technologies are often accompany by unwanted side effects and require high energy and cost. The conventional activated sludge process for chemical oxygen demand (COD) removal, which is an oxygen-dependent biological reaction, needs extensive aeration to provide a substantial amount of oxygen [15]. Sludge bulking, which causes low settling ability after the activated sludge process by unsettled biomass growth, is also a major problem since a longer biomass settling time can cause extension of the hydraulic retention time [10]. Additionally, aerobic oxidation of carbon results in carbon dioxide (CO_2) emission, which may contribute highly to global climate change [26]. Nitrogen, which exists mostly in a form of ammonium (NH_4^+-N) in wastewater, is generally removed by an advanced treatment process after the activated sludge process, using microbiological pathways consisting of aerobic nitrification (NH_4^+-N oxidation to NO_3^--N) and anoxic denitrification (NO_3^--N reduction to N_2). Both pathways are energy consuming owing to oxygen and external carbon source requirements for nitrification and denitrification, respectively.

Compared with the conventional wastewater treatment processes, the use of algal-bacterial consortia can be an

energy effective and environmentally sound method for simultaneous removal of COD and nitrogen [30]. This process is based on symbiotic interactions between algae and bacteria. Briefly, the oxygen produced by algal photosynthesis from light and CO_2 is used by bacteria to oxidize COD and nitrogen in the wastewater [30]. The CO_2 released by the bacteria is then consumed by the algae, and nitrogen is synthesized in the algal biomass from photosynthesis. This cyclic process can efficiently remove COD and nitrogen from wastewater as well as mitigate greenhouse gas emissions [31]. During the algal-bacterial process, organic COD (*i.e.*, existing COD in wastewater and COD released from algal growth) can be oxidized by only bacteria, whereas both algae and bacteria are capable of nitrogen removal under oxic condition [22]. Algae takeup nitrogen such as ammonium (NH_4^+-N) and nitrate (NO_3^--N) to synthesize biomass [8]. Nitrifying bacteria present in municipal wastewater can oxidize NH_4^+-N to NO_3^--N, which can be used as an electron acceptor in the subsequent denitrification process [36]. Thus, algae and bacteria could be reasonably paired for an efficient nitrogen removal process.

Symbiotic algal-bacterial COD and nitrogen removal in wastewater by algal inoculation has been reported previously. Algal-bacterial biofilms (*i.e.*, *Chlorella vulgaris* and *Azospirillum brasilense*) achieved up to 90% COD removal and complete removal of ammonium in municipal wastewater [1]. Oxygen produced by algae supports bacterial nitrification and enhances nitrogen removal [17]. Specific bacterial species were introduced to promote fast growth of algal species for 78~91% of nitrogen removal [6]. On the other hand, studies of algal-bacterial competitions have also been introduced. Algal metabolites possibly influence bacterial growth and change the aquatic ecology [22]. The toxicity of algal metabolites (*i.e.*, kawaguchipeptin, calothrixine, and 12-epi-hapalindole E) inhibits bacterial processes, including nitrifying bacteria as well as other heterotrophs, and vice versa, some bacteria that can produce algicidal metabolites (*i.e.*, 2-hydroxy-12-oleanene-3, 28-O-D-glucopyranosyl, L-histidine, *o*-tyrosine, *N*-acetylhistamine, and urocanic acid) are known to inhibit algal growth [24, 39].

Inoculation of specific algal species into wastewater to improve COD and nitrogen removal and the performance of algal-bacterial consortia has been reported. Specifically, *Chlorella*, *Selenastrum*, and *Scenedesmus* are known for effective nutrients removal. In addition, these genera have been isolated from wastewater; thus, they were being widely applied for nitrogen removals in wastewater [25, 33, 38]. *Chlorella* sp. is a unicellular algae and is popularly known as a fast-growing algal genus to remove ammonium by over 95% in wastewater [6, 12, 19]. *Selenastrum* sp. and *Scenedesmus* sp. have also been reported to be widely used for various kinds of wastewater treatment (*i.e.*, swine wastewater and municipal wastewater), achieving approximately 85% of total nitrogen (TN) removal [7, 9, 18, 25].

Although the algal-bacterial-based wastewater treatment process can be a promising approach for simultaneous removal of COD and nitrogen and for reduction of greenhouse gas emission from wastewater treatment facilities, the indigenous bacterial dynamics affected by inoculation of different algal species into wastewater still remain unknown. Hence, investigations on how a specific algal species affects the indigenous microbial community and their functions should be preceded to improve wastewater treatment performance.

The objectives of this study were to characterize COD and nitrogen removal, and the dynamics of the indigenous bacterial community in response to inoculation of different algal species into municipal wastewater. Indigenous algae in municipal wastewater were filtered to see the effect of each inoculated algal species independently. *Chlorella vulgaris*, *Selenastrum gracile*, and *Scenedesmus quadricauda* were selected for inoculation into the algae-free wastewater.

Materials and Methods

Algal-Bacterial Cultures

Municipal wastewater as the natural medium for algal growth was obtained from Seo-Nam Wastewater Treatment Plant, Seoul, South Korea. Table 1 shows the characteristics of the collected municipal wastewater. The municipal wastewater samples were filtered using 1.2 μm glass microfiber filters (GF/C filters; Whatman, UK) to remove indigenous algae, but retain the indigenous bacteria in the wastewater. *Chlorella vulgaris* AG10032, *Selenastrum gracile* UTEX 325, and *Scenedesmus quadricauda* AG 10308 were selected to construct the algal-bacterial consortia in this study. These algal species were provided by the Korea Research Institute of Bioscience and Biotechnology in Daejeon, South Korea.

The algal species were added into 6 L bottles (DURAN Group, Germany) containing 5 L of algae-free filtered wastewater. Unfiltered

Table 1. Characteristics of influent of municipal wastewater.

Component	Concentration (mg/l)
BOD	120.8
COD	61.5
SS	116.9
TN	32.2
TP	3.90

wastewater that contained indigenous algae was used as a control. Four completely mixed batch reactors were operated at 25–26°C for 9 days under consistent photoreactive conditions (132 μE light intensity). The initial algal chlorophyll *a* (Chl-*a*) concentration of all reactors was set as 10 μg/l. During the incubation, samples were periodically collected from the reactors for further biological and chemical analyses.

Chlorophyll *a* Measurement

To monitor algal growth, Chl-*a* was measured using Sartory and Grobbelaar's protocol [32]. The samples were collected from the reactors and filtered using 1.2 μm glass microfiber filters (GF/C filters; Whatman, USA) to collect the algal biomass. Each glass filter was inserted in 15 ml BD Falcon tubes (BD Biosciences, USA), and 7.6 ml of 95% ethanol was added. The tubes were shaken for 20 min in a warm water bath (70–73°C) and subsequently cooled in a box for 3 h. After centrifugation for 2 min at 2,400 rpm, Chl-*a* in the supernatants was analyzed using a T60 UV/Vis spectrophotometer (PG Instruments, Ltd., UK) at 649, 665, and 750 nm. The Chl-*a* concentrations were calculated using the following empirical equation [13]: Chl-*a* = $[13.7(A_{665} - A_{750}) - 5.76(A_{649} - A_{750})] \times E/F \times l$, where Chl-*a* = concentration of chlorophyll *a* (μg/l); E = volume of ethanol (ml); l = cuvette path length (cm); F = filtration volume (L); and A_i = absorbance at wavelength *i*.

Chemical Analyses

The chemical oxygen demand, total nitrogen, and ammonium (NH_4^+-N) were analyzed after filtration using 0.2 μm GF/C filters (Whatman, UK) and measured in a spectrophotometer (DR4000; HACH, USA) with HACH reagents. The COD was measured using the COD Digestion Reagent (HACH). TN and NH_4^+-N and were measured using the Total Nitrogen Reagent Set (HACH) and the Nitrogen-Ammonia Reagent Set (HACH), respectively.

DNA Extraction and Quantitative Real-Time PCR

Genomic DNA was extracted from each sample using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, USA). Quantitative real-time PCR (qPCR) was performed using the Bio-Rad iQ5 real-time PCR detection system (Bio-Rad, USA). For PCR amplifications, reaction mixtures contained 10 μl of 2× iQ SYBR Green Supermix (Bio-Rad), 0.6 μl of each primer (10 mM), and 20 ng of template DNA in a volume of 20 μl. Triplicate experiments were carried out, with negative controls containing no template DNA. The primer set targeting bacterial 16S rRNA genes was 1055F (ATG GYT GTC GTC AGC T) and 1392R (ACG GGC GGT GTG TAC) [11]. The thermocycling steps were (i) 95°C for 10 min; (ii) 40 cycles at 95°C for 15 sec, 52°C for 1 min, 72°C for 20 sec; (iii) 80°C for 15 sec; and (iv) 1 cycle at 95°C for 15 sec, 60 to 95°C for melting curves.

Bacterial Community Analysis

To analyze the bacterial community dynamics in response to different algal species, 16S rRNA gene-based terminal-restriction fragment length polymorphism (T-RFLP) and cloning-Sanger sequencing methods were used. For T-RFLP analysis, bacterial 16S rRNA genes were amplified using a fluorescent dye-bound forward primer, 27f-FAM (6-FAM-AGA GTT TGA TCA TGG CTC AG), and reverse primer, 1492r (TAC GGT TAC CTT GTT ACG ACT T) [35]. For PCR amplification, 10 μM 27f-FAM and 1492r primer solution (0.5 μl), 5 U/μl *Taq* polymerase (0.25 μl) (Fermentas, Germany), and 20 ng template DNA were used in a total volume of 25 μl. PCR was performed using the 1000 Thermal Cycler (Bio-Rad) with the following thermal program: (i) 94°C for 3 min; (ii) 30 cycles at 94°C for 1 min, 55°C for 30 sec, and 72°C for 2 min; and (iii) 72°C for 10 min. The amplified products were purified using a QIAquick PCR Purification Kit (Qiagen, Inc., USA). The purified amplicons were digested at 37°C for 4 h using the restriction enzyme HhaI. For the digestion, each reaction (20 μl) contained 10 U HhaI, Tango buffer, and distilled water. After purification of the digested PCR products using the QIAquick PCR Purification Kit, restriction fragment length polymorphisms (RFLPs) were analyzed using an ABI 96-Capillary 3730xl DNA Analyzer (Applied Biosystems, USA). T-RFLP profiles were produced using Gene Mapper Software ver. 4.0 (Applied Biosystems, USA). Terminal restriction fragments (T-RFs) with a peak lower than a 10 standard deviation threshold value were considered as analytical noise and removed from the profiles. Nonmetric multidimensional scaling (NMDS) analysis was performed using R and Vegan package with Bray-Curtis dissimilarity metrics to compare the T-RFLP profiles.

For the cloning-Sanger sequencing, bacterial 16S rRNA genes were amplified as described for the T-RFLP method; however, the forward primer did not contain any fluorescent dye. After a purification using a QIAquick PCR Purification Kit (Qiagen), the 16S rRNA gene amplicons were ligated with the pCR 4-TOPO TA vector using a TOPO TA Cloning Kit for Sequencing (Invitrogen, Inc., USA) following the manufacturer's instruction. Forty-eight purified colonies were selected for each sample and sequenced by Macrogen, Inc. (Seoul, Korea). The sequences were classified using EzTaxon (http://www.ezbiocloud.net/eztaxon) [21]. The 16S rRNA sequences were deposited in GenBank with accession numbers from KT900337 to KT900526.

Results

Effect of Algae Species on Nitrogen Removal

The initial concentrations of TN and NH_4^+-N in the reactors were 32.0 ± 2.8 mg/l and 24.5 ± 0.7 mg/l, respectively. Fig. 1 shows the time-dependent variation of TN and NH_4^+-N concentrations. From the startup of reactor to day 5, the TN concentration was gradually reduced with time as it decreased to 5.0 mg/l, 4.0 mg/l, 13.5 mg/l, and 20.0 mg/l in the reactor grown with *C. vulgaris*, *S. gracile*, *Sc. quadricauda*, and indigenous algal population, respectively (Fig. 1A). The reactors grown with *C. vulgaris* and *S. gracile* exhibited more

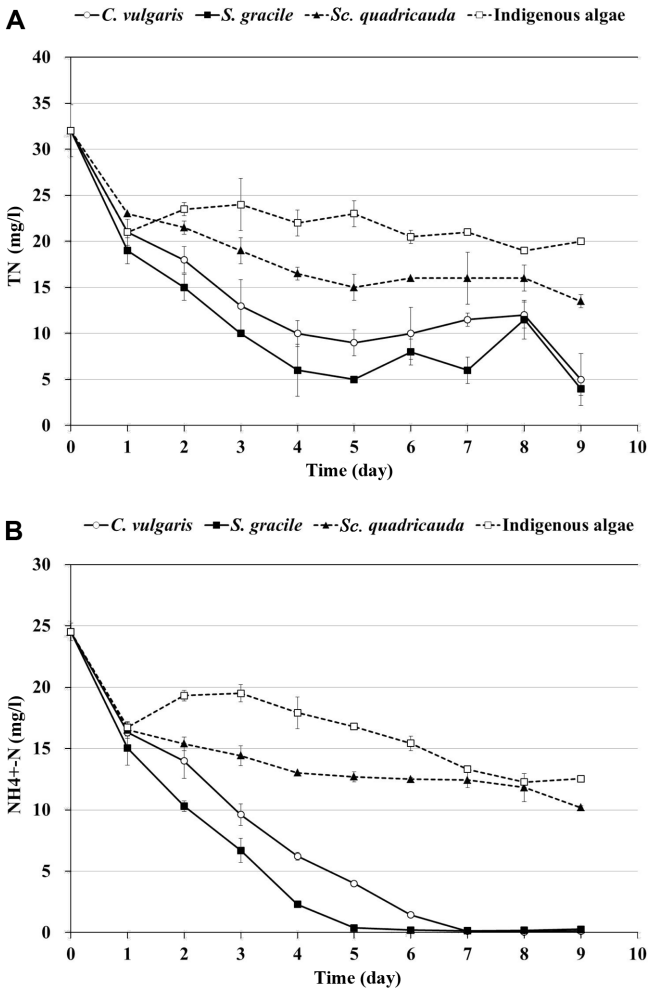

Fig. 1. Time-course profiles of nitrogen removal in response to algal populations ((**A**): TN); (**B**): NH_4^+-N).
Error bars represent standard deviations of three measurements.

effective TN removal rates than those with *Sc. quadricauda* and the indigenous algal populations. Complete NH_4^+-N removal was shown within day 9 in the reactors with *C. vulgaris* and *S. gracile*, whereas NH_4^+-N concentrations were slowly reduced in the range 10–15 mg/l in the reactors with *Sc. quadricauda* and indigenous algal population (Fig. 1B). This finding is consistent with the results of TN removal.

Effect of Algae Species on Soluble COD Removal

The initial soluble COD (sCOD) concentration was 60.8 mg COD/l, which decreased until day 4 and then rebounded to go beyond the initial concentration, except in the reactor with *Sc. quadricauda* and the indigenous algal population (Fig. 2). The sCOD concentration in the reactor with *Sc. quadricauda* remained in the range 25–30 mg/l. The

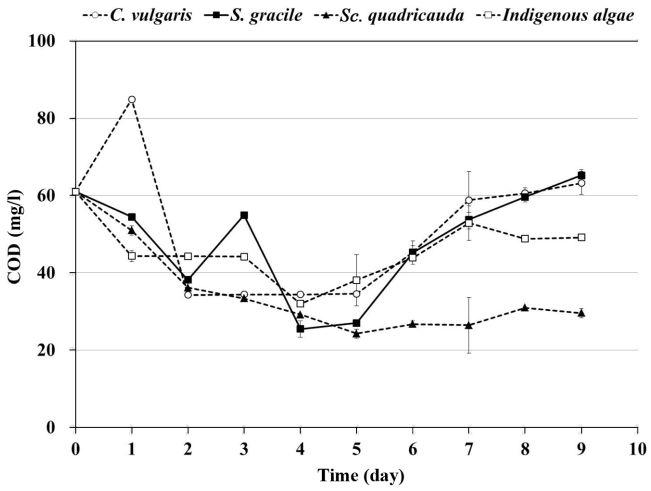

Fig. 2. Time-course profiles of COD removal in response to algal populations.
Error bars represent standard deviations of three measurements.

period of sCOD concentration rebound corresponded to the period when ammonia was fully removed and the TN removal rate was dramatically reduced in the reactor with *C. vulgaris* and *S. gracile*.

Algal and Bacterial Growth

In order to gain more insight into the effect of algae species on carbon and nitrogen removal, both algal and bacterial growth was measured (Figs. 3 and 4). As shown in Fig. 3, the concentration of Chl-*a* in the reactor with *C. vulgaris* and *S. gracile* increased exponentially until day 5, and no more growth of both algae was observed after day 5. A stable number of *Sc. quandricauda* was maintained during the incubation without any further growth, but we could observe the indigenous algal population growing slowly and gradually. It is worth noting that *C. vulgaris* and *S. gracile* stopped growing when ammonium was depleted, and the concentration of sCOD increased in the reactors. It clearly demonstrated that the ammonia concentration is strongly linked with algal growth as a major limiting factor and sCOD increase in the reactor.

The 16S rRNA genes were quantified using qPCR throughout the incubation period (Fig. 4). As shown in Fig. 4, bacterial 16S rRNA gene copy numbers at day 1 varied in a range of 2.1×10^7 to 1.6×10^8 gene copies l^{-1} in all reactors. The bacterial 16S rRNA gene copy numbers in the reactor grown with *C. vulgaris* quickly increased until day 5, while the abundance in the other reactors did not increase significantly. After day 5, the abundance of bacteria decreased rapidly in the reactor with *C. vulgaris* and

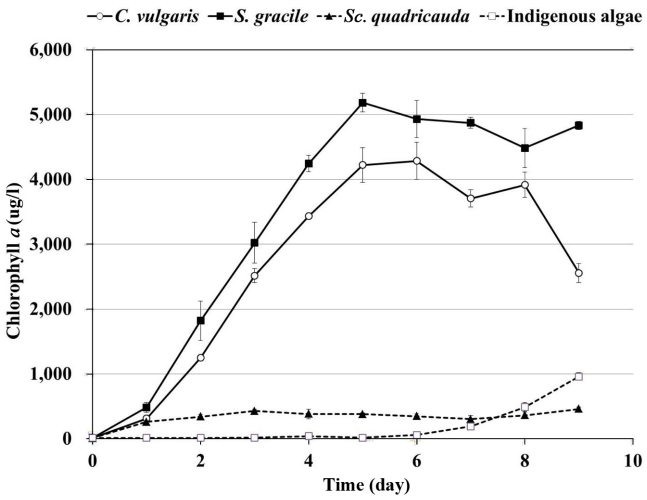

Fig. 3. Time course profiles of algal growth by chlorophyll *a* measurement.
Error bars represent standard deviations of three measurements.

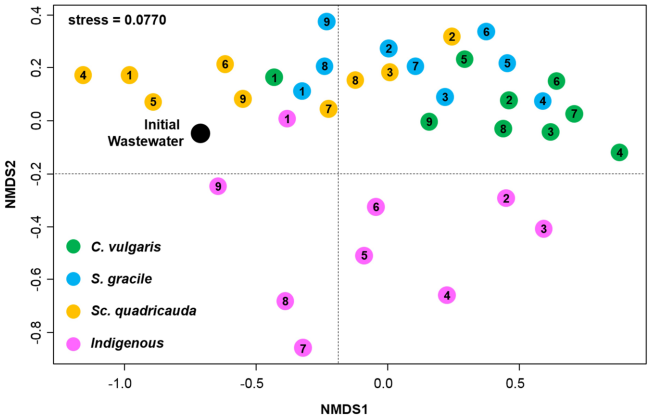

Fig. 5. Non-metric multidimensional scaling (NMDS) plots of T-RFLP from 16S rRNA gene sequences.
The correlations of each sample were calculated by the Bray-Curtis dissimilarity metric. The number in the circle indicates the incubation period (days).

S. gracile to 1.9×10^7 and 7.8×10^5 gene copies/l, respectively, which corresponded to the period when the ammonia concentration was depleted and sCOD increased. It indicates that the ammonia and sCOD concentration could serve as crucial triggers that influence the interaction between algae and bacteria in the consortia.

Bacterial Population Dynamics in Response to Algal Species

The plot for the NMDS analysis of T-RFLP demonstrates the bacterial community differentiation over the incubation period with different algae species (Fig. 5). The bacterial communities in all reactors at day 1 had relatively concentrated distribution, close to the community in the initial wastewater. However, the type of algae species in the reactors exerted significant effects on the bacterial communities after day 1, as spread in the top right area of the ordination plot. These results indicate that the differentiation of bacterial communities in the presence of algae species was strongly influenced by the algae species introduced in the reactor. Generally, the bacterial communities in response to the inoculated algae species were less spread than the community with the indigenous algae population. The fast-growing algal populations (*C. vulgaris* and *S. gracile*) produced roughly similar community structures and were highly concentrated over the incubation periods, but the slow-growing algal population (*Sc. quadricauda*) was relatively distant from the other two species. However, the bacterial population showed spreading out in the bottom of the ordination plot in the presence of the indigenous algal population, supporting that the selection force of bacterial communities was limited as shown in the reactor with inoculation of specific algae species.

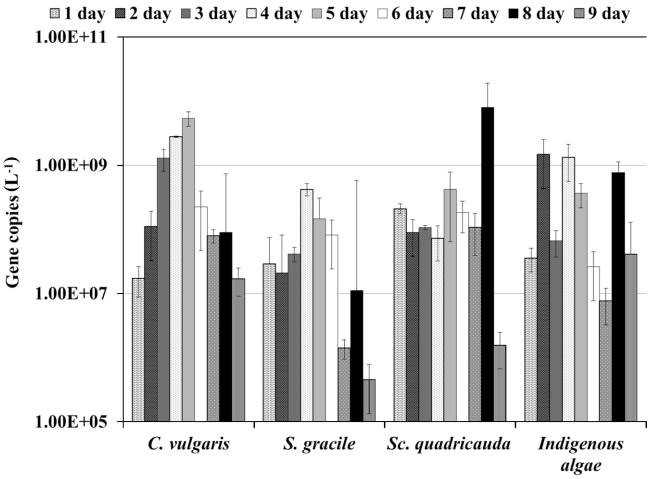

Fig. 4. Time-course profiles of 16S rRNA gene copies in response to algal populations.
Error bars represent standard deviations of the three independent PCRs of the DNA.

To gain insights into the bacterial community differentiations, 16S rRNA gene clone libraries were constructed (Fig. 6). Pearson's correlation coefficient was tested to compare the bacterial communities present in each reactor. Overall, the comparison of the community structures showed significant differences from each other (p-value < 0.05). This finding indicates that the presence of the different microalgae species exerts a strong selection on the bacterial community.

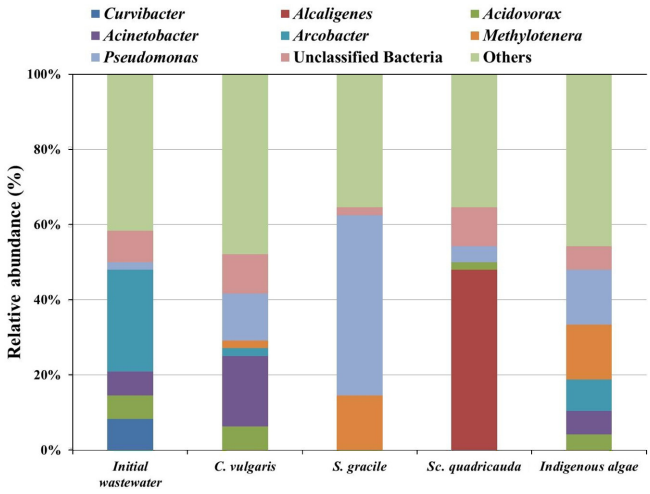

Fig. 6. Classification of bacterial community structures in response to algal populations.
The clone libraries of 16S rRNA genes consisted of 48 clones from each sample.

The sequences related to the genera *Acinetobacter* (18.5%), *Pseudomonas* (43.8%), *Alcaligenes* (45.8%), and *Pseudomonas* (14.5%) were dominant in the reactor with *C. vulgaris*, *S. gracile*, *Sc. quadricauda,* and the indigenous algal populations, respectively.

Discussion

In this study, we investigated whether inoculation of different algal species affects the COD and nitrogen removal efficiency and bacterial community dynamics in municipal wastewater. The reactors that received *C. vulgaris* and *S. gracile* showed better performance for COD and TN removal than the reactor with *Sc. quadricauda*, until the ammonium was depleted. We also found that the bacterial community dynamics were strongly dependent on the inoculated algal species.

C. vulgaris and *S. gracile* were found to be fast growers in the wastewater reactors, while *Sc. quadricauda* grew slower. Reactors inoculated with the fast growers exhibited high efficiencies of nitrogen removal (83.3% and 86.6% of TN removal for *C. vulgaris* and *S. gracile*, respectively) during the incubation periods until day 5 compared with the slow grower. The increased algal and bacterial biomass was accompanied by ammonium decrease during the first five days. It indicates that both algae and bacteria takeup ammonium for biomass production and as an energy source, and then promote nitrogen removal in both reactors. These observations suggest that the interaction between algal and bacterial populations enhanced the nitrogen removal under sufficient N availability and light source. Several studies have supported that the algal population may stimulate bacterial nitrification by increasing oxygen availability to bacteria via oxygenic photosynthesis [17]. Since ammonium was not limiting algal and bacterial growth until day 5, the efficiency of TN and ammonium showed similar trends over time. An immediate decrease in the growth of both *C. vulgaris* and *S. gracile* at day 5 was clearly observed and coincided with ammonium depletion. Previous studies have shown the inhibition of algal growth associated with ammonium limitation [20].

Given that direct competitive interaction occurred between algae and bacteria during the nitrogen removal, both the algae and bacteria must grow slowly owing to competition of algal N uptake and bacterial ammonia oxidation [4]. Our results may support the competition theory in the reactor with slow-growing algae species (*Sc. quadricauda*), where both algal and bacterial populations grew slowly compared with fast growers even though there was no limiting nutrient factors, including total phosphorus, which was present as 2.0 mg/l in the reactor of *Sc. quadricauda* (data not shown). The efficiency of nitrogen removal, including TN and ammonium, was considerably lower than those in reactors with a fast grower. Even though *Sc. quadricauda* is well known as a nitrogen removing species in municipal wastewater, the reason for its slow growth might be related to indigenous *Alcaligenes*, which was dominant in a *Sc. quadricauda*-inoculated reactor especially and known as an algicidal bacterium [27]. In addition, *Alcaligenes* is known as a heterotrophic nitrifying and aerobic denitrifying bacterium, and it suggests that the slow ammonium removal in the *Sc. quadricauda*-inoculated reactor might be due to nitrification and denitrification by the *Alcaligenes* population [16].

The decrease of bacterial gene copies after day 5 in the reactors with *C. vulgaris* and *S. gracile* can be explained from the results of COD and ammonium. After this immediate response to ammonium limitation in the reactor with fast growers, the concentration of COD rebounded remarkably up to the initial concentration. Increased COD might mainly consist of algal metabolites that can be produced under the nitrogen limited condition [28] and may contain toxic compounds to inhibit bacterial growth. The results are supported by previous studies reporting the inhibition of bacterial growth by algal metabolites [23, 34]. In addition, ammonium could be dependent on the bacterial abundance. As mentioned about the possibility of bacterial nitrification in this study, bacteria might not grow at the condition of

ammonium depletion.

To examine how the bacterial community structure changed in response to algal population over time, NMDS plots were constructed (Fig. 5). We observed clearly the community influenced by external inoculation including *C. vulgaris*, *S. gracile*, and *Sc. quadricauda*, seperate from the indigenous algal population. As the incubation period approached day 9, the bacterial communities in the reactor with fast growers (*C. vulgaris* and *S. gracile*) converged together, while with the slow grower (*Sc. quadricauda*) and indigenous algal population they were spread out. Fast growers seemed to exhibit a stronger selection effect than the slow grower, implied from the tight clustering of bacterial community in the reactor with the fast grower in the NMDS plot up to day 9. On the other hand, NMDS ordination places a slight shift of bacterial communities from fast growers more distantly from each other as time progresses. It seemed that this slight shift accentuated the effects of specific algal growth on the bacterial communities even in fast growers.

Discrepancies in community shifts in response to algal species could be explained by specific metabolites from different algal species. The types and production rate of metabolites differ with algal species [3]. The characterization of metabolites produced from each algal species was not made in this study, since the screening of total metabolites using conventional technology is so difficult and limited in discovering a certain compound associated with specific bacteria in the population [37]. However, the difference in bacterial community investigated by 16S rRNA clone libraries between inoculation algal types and indigenous algal population was generally apparent over the incubation periods. These observations suggest that the bacterial population might be linked to metabolites produced from algal species. At the onset of inoculation, dissimilarity in bacterial community shifts could be initially influenced by competition with nitrogen sources and, later, with the nitrogen depletion, also influenced by metabolites from algal populations.

The dominant bacterial members in the reactor seemed to be adapted to the algal populations. Hence, the different members in response to each algal population may be indicative of symbiosis mechanisms that exchange the compounds for biomass or energy production [34]. The convincing evidence for specific algal-bacterial interaction in this study comes from identification of heterotrophic nitrifying bacteria as dominant members in the reactors. *Acinetobacter* and *Pseudomonas*, which were the most dominant in response to *C. vulgaris* and *S. gracile*, respectively, are known heterotrophic nitrifying bacteria [5, 14]. This supports that bacterial nitrification and algal nitrogen uptake can occurr simultaneously. *Alcaligenes*, which was dominant in the *Sc. quadricauda*-inoculated reactor, is also a known heterotrophic nitrifying bacterium [16].

Given that there is a considerable amount of previous studies on nitrogen and carbon removal by algal and bacterial consortia, the present work focused specifically on the impact of specific algal populations on wastewater treatability and bacterial community dynamics. The use of municipal wastewater and an indigenous bacterial population shows a more feasible representation of the wastewater treatment and bacterial response to different algal species in the wastewater treatment plants. Various kinds of indigenous bacterial populations in municipal wastewater could be cooperative or competitive against algal inoculation. In this study, interacting bacterial populations were selected and characterized from algal species well-known for COD and nitrogen removal in municipal wastewater. This further suggests that the controlling factors for balanced enrichment of indigenous bacteria to symbiotically interact with algal species in the algal-bacterial treatment processes are to control COD and N removal simultaneously.

Acknowledgments

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