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Effect of Particle Property on Coagulation and Degradation of Residual Rubber in Natural Rubber Wastewater

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Abstract

In natural rubber (NR) wastewater treatment, the removal of residual rubber is necessary to apply a closed anaerobic system for the reduction of greenhouse gas emissions. In this study, the effect of particle properties on residual rubber removal was evaluated by a lab-scale wastewater treatment system of NR wastewater. In NR wastewater, major particle sizes of residual rubber were more than 1.0 µm, 0.45–0.1 µm and less than 0.1 µm. At an early stage of the PTC, smaller particle sizes of residual rubber were reduced, while middle particle sizes were increased. In contrast, at a later stage of the PTC with increasing biodegradability, larger particle sizes of residual rubber were reduced, while smaller particle sizes of residual rubber were increased. As residual rubber removers, *Acinetobacter* and *Pseudomonas* are detected for biological coagulation and degradation of residual rubber in the PTC, respectively. In contrast, *Gordonia* was detected as the predominant rubber degrader in the DHS reactor. The early and later stages of the PTC function as rubber coagulation and rubber degradation stages, respectively. Furthermore, *Pseudomonas* and *Gordonia* are considered degradation bacteria for liquid rubber and solid rubber, respectively.

1. Introduction

The natural rubber (NR) industry is one of the major agro-industries in South-eastern Asia. NR is considered a carbon-neutral material. In the life cycle of NR, major emissions of greenhouse gases (GHGs) occur during wastewater treatment, which is done in open-type anaerobic and aerated tanks. Major GHGs emitted during wastewater treatment are methane and nitrous oxide, which are emitted from the anaerobic tank [1], and carbon dioxide derived from power consumption for aeration in the aerated tank. To reduce GHG emissions in the wastewater treatment system, the application of a closed anaerobic reactor and a non- or less-aerated aerobic reactor is appropriate. The application of a closed anaerobic reactor can contribute to the reduction of GHG emissions in the wastewater treatment system, as well as the processing of NR products, by utilising biogas as an energy source. For the application of a non-aeration aerobic reactor, a trickling filter can be considered. However, residual rubber in NR wastewater causes clogging of the closed anaerobic reactor by coagulation, and it reduces the process performance of the trickling filter by accumulating at the surface of the biomass carrier. Therefore, an appropriate pre-treatment process for residual rubber removal is required.

In our previous study, we proposed the pre-treatment canal (PTC), which is the drainage canal equipped with lumps of block rubber (core rubber) as the pre-treatment process for residual rubber removal, and combined with an anaerobic baffled reactor (ABR) and down-flow hanging sponge (DHS) reactor system (ABR-DHS system) [2]. Both coagulation and degradation of residual rubber were confirmed in the PTC. Furthermore, the PTC contributed to enhancing methane recovery in the ABR and nitrogen removal in the DHS reactor by degrading residual rubber into low-molecular substances and preventing rubber accumulation in the DHS reactor, respectively. The key factors for the coagulation of residual rubber in the NR wastewater were reported to be contact with the core substance and air [3]. However, the coagulation and degradation mechanisms of residual rubber remain unclear.

The DHS reactor, which is a trickling filter equipped with sponges as biomass carriers, was applied mainly for nitrogen removal in wastewater treatment of NR processing factories [4], [5]. In NR wastewater treatment using the DHS reactor, rubber accumulation at the surface of the sponge carriers was confirmed. Additionally, *Gordonia*, aerobic rubber-degrading bacteria, were highly detected [3]. Therefore, accumulated rubber was considered as a carbon source for denitrification in the DHS reactor.

In this study, we evaluate the property effect of NR latex particle size on coagulation and degradation in NR wastewater treatment by changing the particle size distribution of residual rubber in the PTC. Furthermore, we consider the applicability of core rubber for the enhancement of rubber recovery, rubber degradation, and nitrogen removal.

2. Materials and Methods

2.1 Experimental Setup

Fig. 1 shows a schematic of the wastewater treatment system used in this study. The system includes a pre-treatment PTC and a main treatment ABR-DHS system. The PTC, the ABR, and the DHS reactor were made of polyvinyl chloride pipes. The PTC (effective volume: 1.9 L) was equipped with core rubbers, which are accumulated in the DHS reactor during the treatment of NR wastewater [2], as a core substance for rubber coagulation. To enhance rubber removal in the PTC, a 0.5 L of polyethylene terephthalate bottle equipped with core rubber was installed at the influent part of the PTC on operating day 133. Before and after operating day 133 were set as Phases 1 and 2, respectively. The ABR (effective volume: 8.5 L) and the DHS reactor (effective volume: 4.0 L) each have four compartments and three boxes. The polyurethane sponges were used as biomass carriers in the DHS reactor. The hydraulic retention times (HRTs) of the PTC, ABR, and DHS reactor were 1.0, 4.5, and 2.1 days, respectively. A part of the DHS effluent was recirculated into the fourth compartment of the ABR via a desulfurization trickling filter, which was equipped with coconut chips as contact material, at a recirculation ratio of 1.0. The system was installed in a greenhouse set at 30°C.

The synthetic NR wastewater was obtained by mixing NR latex, acetic acid, propionic acid, and ammonia, based on a previous study [1]. The synthetic wastewater contains 6,400 ± 1,340 mg/L, 2,220 ± 660 mg/L, 366 ± 208 mg/L, 168 ± 38 mgN/L, and 4.82 ± 0.25 of total chemical oxygen demand (COD), total biochemical oxygen demand (BOD), suspended solids (SS), ammonia nitrogen, and pH, respectively. The sodium acetate solution was used as a carbon source for denitrification, and it was fed to the third box of the DHS reactor during Phase 1.

2.2 Chemical Analysis

Water samples were collected twice weekly from the wastewater tank, and PTC, ABR, and DHS effluents. The pH and gas production rate were measured using a pH meter (KP-10Z, KRK, Japan) and a wet-type gas meter (WS-1A, Shimadzu, Japan), respectively. Total COD, ammonia, nitrite, and nitrate concentrations were analysed using a Hach apparatus (DR/2500, Hach, USA) and an ion chromatograph equipped with a conductivity detector (CDD-10A VP, Shimadzu, Japan), respectively. BOD and SS were analysed by a standard method [6].

2.3 Microbial Community Analysis

Analysis of the microbial communities in the sludges retained in the PTC, ABR, and DHS reactor was conducted on day 75 (Phase 1) and day 322 (Phase 2, only for the PTC). DNA extraction was performed using a MonoFas Bacterial Genomic Kit IV (GL Sciences, Tokyo, Japan). Polymerase chain reaction (PCR) amplification of the 16S rRNA gene [7] was performed. The PCR products were then purified using AMPure XP SPRI beads (Beckman). Massive parallel 16S rRNA gene sequencing was conducted using the Miseq system (Illumina) and a Miseq Reagent Kit v.3. Analysis of sequential data [8] was conducted using the QIIME software package v.1.9.1. Furthermore, the operational taxonomic units were classified at a 99% sequence identity level. The SILVA database was used to perform the taxonomic classification [9] for the 16S rRNA gene.

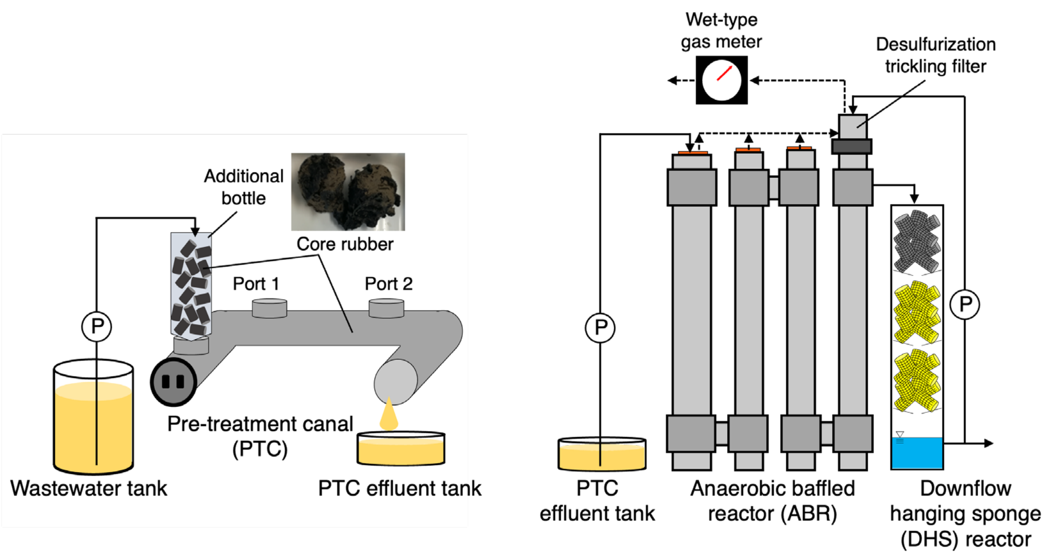

Fig. 1 *Schematic of the wastewater treatment system: (a) Pre-treatment canal (PTC), and (b) Anaerobic baffled reactor (ABR) and a down-flow hanging sponge (DHS) reactor system (ABR-DHS system)*

3. Results and Discussion

3.1 Rubber Removal in The PTC

Fig. 2 shows the average SS concentrations of wastewater and PTC effluent during Phases 1 and 2. In the PTC, COD removal efficiency increased from 10.3% to 21.9% (from Phase 1 to 2). However, SS concentration was increased in the PTC. The particle size range of NR latex was reported to be from 0.04 to 4 μm [10]. Thus, the smaller NR latex particle size was increased to more than 0.45 μm, which was accounted as SS, in the PTC. Following continuous operations, the growth rate of SS decreased from 31.9% to 4.5%. Additionally, the collected amount of residual rubber also increased from 0.17 kg/m^3-wastewater (Phase 1) to 0.27 kg/m^3-wastewater (Phase 2). The collected amount in the additional bottle shared 18.7% of the total amount in Phase 2. These results indicated that the coagulation of residual rubber was enhanced by overgrown core rubber in the PTC. Additionally, tickling filter-type core rubbers had a better performance for the coagulation of residual rubber than immersed-type core rubbers. However, the collected amount of residual rubber in this study was approximately 1/10 of that of our previous study [2]. The SS concentration of the wastewater in this study was less than 1/4 of that of our previous study [2]. Therefore, it was considered that the particle size of latex affected the coagulation and degradation of residual rubber in NR wastewater.

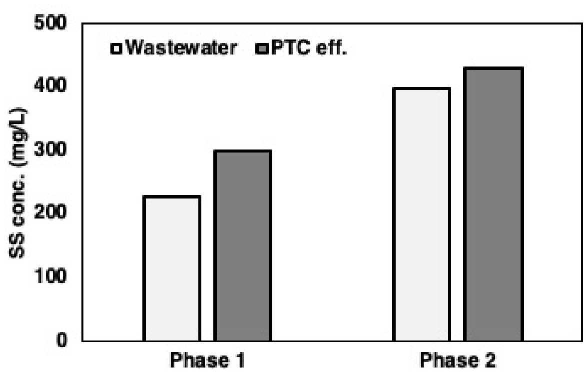

Fig. 2 *Average SS concentration of wastewater and PTC effluent*

Fig. 3 shows the particle size distribution of residual rubber in the PTC during Phase 2. The particle size distribution was calculated by the COD concentration of the filtrates for each size of the filter. The removed part was calculated by the total COD concentration of wastewater and each sampling point. In the wastewater, the particle sizes of more than 1.0 μm, 0.45–0.1 μm, and less than 0.1 μm were shared at 25.4%, 26.9%, and 45.2%, respectively. Acetic and propionic acids shared 85% of the COD concentration of the particle size of less than 0.1

μm (38.4% of total COD). From influent to Port 1, 23.9% of COD was removed in the PTC. Within this interval, particle sizes of 0.45–0.1 μm and less than 0.1 μm were reduced to 12.5% and 33.5%, respectively. However, the particle size of 1.0–0.45 μm was increased approximately multiply. These results indicated that the particle size of residual rubber was increased, and most of the removed COD was considered as coagulation and accumulation of residual rubber in the PTC. In contrast, the particle size of more than 1.0 μm decreased from Port 2 to the effluent. Then, the particle sizes of 0.45–0.1 μm and less than 0.1 μm were increased. Within this interval, BOD concentration was increased from 1,630 to 1,720 mg/L, even though COD concentration was decreased from 5,730 to 5,220 mg/L. These results indicated that the biodegradability of wastewater was increased by the degradation of high molecular weight organic matters, such as residual rubber, to low molecular weight compounds with longer HRT. Therefore, the early and later stages of the PTC functioned as rubber coagulation and rubber degradation stages, respectively.

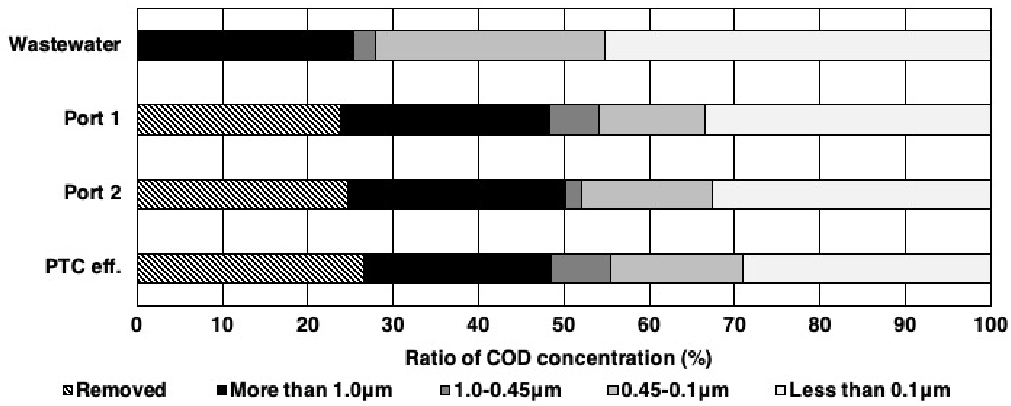

Fig. 3 *Particle size distribution of residual rubber in the PTC*

3.2 Organic Matters and Nitrogen Removal in The ABR-DHS System

Fig. 4 shows the average COD and BOD concentrations of each sampling point during Phase 2. In the ABR, 81.8 ± 7.7% and 84.4 ± 8.9% of COD and BOD, respectively, were removed. Compared with our previous studies [2], [3], [11], the highest COD removal efficiency of the ABR was achieved in this study. Additionally, the BOD/COD ratio of the ABR effluent was 30.5%, which was three times higher than that of the ABR effluent treating pre-treated NR wastewater by the PTC [2]. In this study, the HRT of the PTC was increased from 1.3 hours to 1.0 days compared with that of the previous study. Most rubber-degrading bacteria were reported as aerobic bacteria [12], [13]. Therefore, residual rubber was degraded to intermediates, which can be caused by anaerobic bacteria, in the PTC with longer HRT. Then, these intermediates were degraded into low-molecular organic matters, which can be caused by BOD, in the ABR.

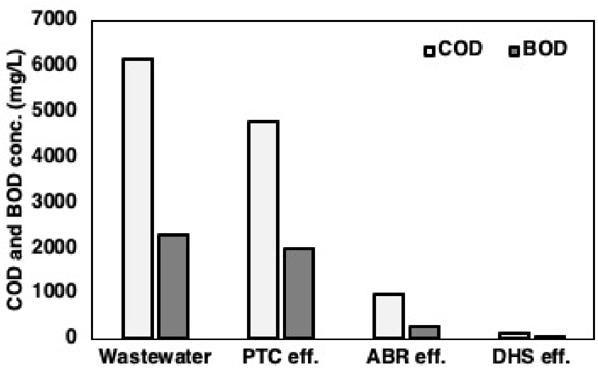

Fig. 4 *Average COD and BOD concentrations of each sampling point*

Finally, 97.8 ± 1.5% and 99.6 ± 0.3% of COD and BOD, respectively, were removed from the ABR-DHS system. In the first and second boxes of the DHS reactor, rubber accumulation was confirmed at the surface of the sponge carriers. Nitrogen removal rates of the DHS reactor with (Phase 1) and without (Phase 2) feeding of sodium acetate solution were 0.078 and 0.082 kgN/(m^3.d), respectively. These values were similar to DHS reactors treating anaerobically treated NR wastewater with residual rubber accumulation [3], [11], but they were lower than DHS reactors without rubber accumulation [2], [4], [5], [14]. Additionally, 44.7% and 21.2% of the inlet ammonia remained as ammonia and nitrate, respectively, in the DHS effluent during Phase 2. Therefore, rubber accumulation at the surface of the sponge carriers inhibited the nitrification performance of the DHS reactor. In contrast, 34.1% of the inlet total inorganic nitrogen was removed in the DHS reactor without feeding sodium acetate solution as a carbon source for denitrification during Phase 2. These results suggest that the accumulated rubber was degraded into intermediates via rubber-degrading bacteria, which were then utilised as carbon sources for heterotrophic denitrification in the DHS reactor. In the solid-phase denitrification process, solid carbons, such as biomass and synthetic polymers, were utilized as a carbon source for denitrification and biomass carrier [15], [16]. Thus, the possible utilisation of accumulated rubber as a solid carbon source for denitrification and a biomass carrier was considered.

Water quality of DHS effluent was 7.97 ± 0.49, 113 ± 75 mg/L, 8 ± 7 mg/L, 71 ± 48 mg/L, 45 ± 18 mgN/L, and 61 ± 16 mgN/L of pH, COD, BOD, SS, ammonia, and total inorganic nitrogen, respectively. These values achieved discharge standards for pH, COD, BOD, and SS in Vietnam, Thailand, and Indonesia, which are major NR-producing countries. However, the discharge standards for ammonia and total nitrogen were not achieved in Vietnam (40 mgN/L of ammonia and 60 mgN/L of total nitrogen) and Indonesia (15 mgN/L of ammonia and 25 mgN/L of total nitrogen). In the DHS reactor, nitrification was a rate-limiting step for nitrogen removal. In this study, the first and second boxes of the DHS reactor functioned as a rubber trap, and then, the nitrification part was decreased. In contrast, accumulated rubber was utilised as a solid carbon source for denitrification in the DHS reactor. Therefore, the increase of DHS's HRT for nitrification by additional boxes was considered as an effective way to enhance nitrogen removal in the DHS reactor and achieve discharge standards.

3.3 Microbial Community in The Wastewater Treatment System

Fig. 5 shows the ratios of the total DNA sequence of the predominant bacteria in the PTC on operating day 75 (Phase 1) and day 322 (Phase 2). In the PTC, *Acinetobacter*, *Desulfovibrio*, and *Pseudomonas* were increased during continuous operation. *Acinetobacter* was highly detected from open-type ABR treating ribbed smoked sheet rubber processing wastewater [17]. At the surface of this open-type ABR, coagulated rubber was confirmed. Additionally, *Acinetobacter* has the ability for biological coagulation of skim latex [18]. In contrast, *Pseudomonas* was known as a degrader of NR [19]. Therefore, these two genera were considered key microorganisms for the coagulation and degradation of residual rubber in the PTC. *Desulfovibrio* is a sulfate-reducing bacterium, and its biosorption ability of zinc was reported [20]. Thus, high detection of *Desulfovibrio* was conducted by zinc in NR latex. *Prevotela*, *Bacteroides*, *Megasphaera*, and *Succiniclasticum* were also predominantly detected. These bacteria can utilise sugars, gluconate, and succinate, and produce organic acids, such as acetate and propionate [21]–[24]. These bacteria were considered to contribute to the hydrolysis and acid production of residual rubber in the wastewater.

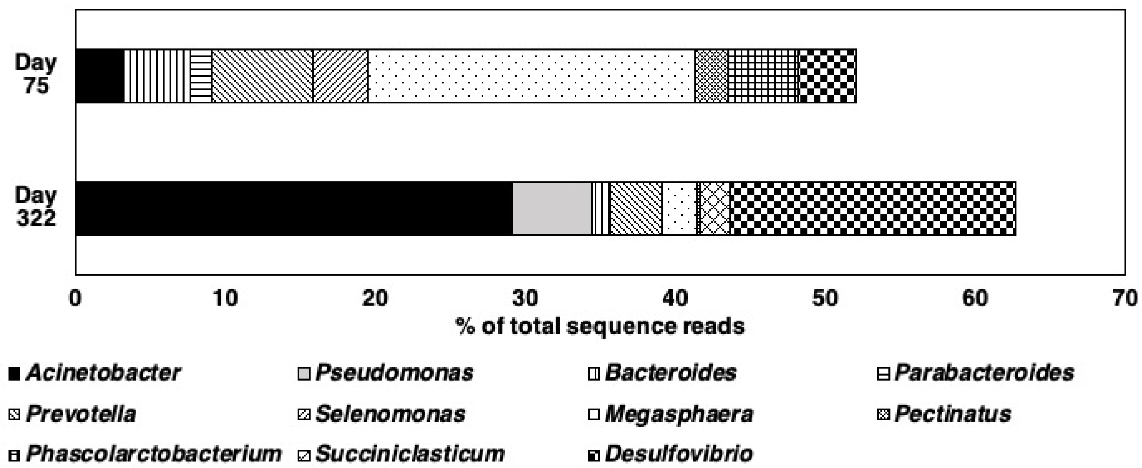

Fig. 5 *Ratio of the total sequence of the predominant microorganisms in PTC on operating day 75 and day 322*

Fig. 6 shows the ratios of the total DNA sequence of the predominant bacteria in the ABR and DHS reactor on operating day 75 (Phase 1). In the ABR, acetate and hydrogen-utilising methane-producing archaea (MPA), *Methanosaeta* and *Methanobacterium*, were predominantly detected, especially in the first and second compartments (C1 and C2). In these compartments, syntrophic organic acids-degrading bacteria: *Syntrophobacter*, *Syntrophomonas*, and *Pelotomaculum*, were also highly detected. Most of the dissolved organic matters in the PTC effluent were acetate and propionate. Therefore, acetate was directly utilised by Methanosaeta, and propionate was utilised under syntrophic association with *Methanobacterium*, *Syntrophobacter*, *Syntrophomonas*, and *Pelotomaculum* at C1 and C2. In contrast, fructan-utilising bacteria, *Treponema*, and protein-degrading bacteria, *Porphyromonas*, were increased in the third and fourth compartments (C3 and C4). The major end products of *Treponema* and *Porphyromonas* were organic acids, such as formate, acetate, propionate, and butyrate [25], [26]. In our previous study, the detection rate of MPA at C3 and C4 was higher than that at C1 and C2 in the ABR treating NR wastewater without the PTC [3]. These results indicated that low-molecular-weight organic matters in the NR wastewater were degraded into organic acids in the PTC and contributed to enhancing methane production at the early stage of the ABR. Furthermore, the remaining carbohydrate and protein were degraded with longer retention times at the later stage of the ABR. Then, the ABR effluent still contained biodegradable organic matter with a 30.5% BOD/COD ratio.

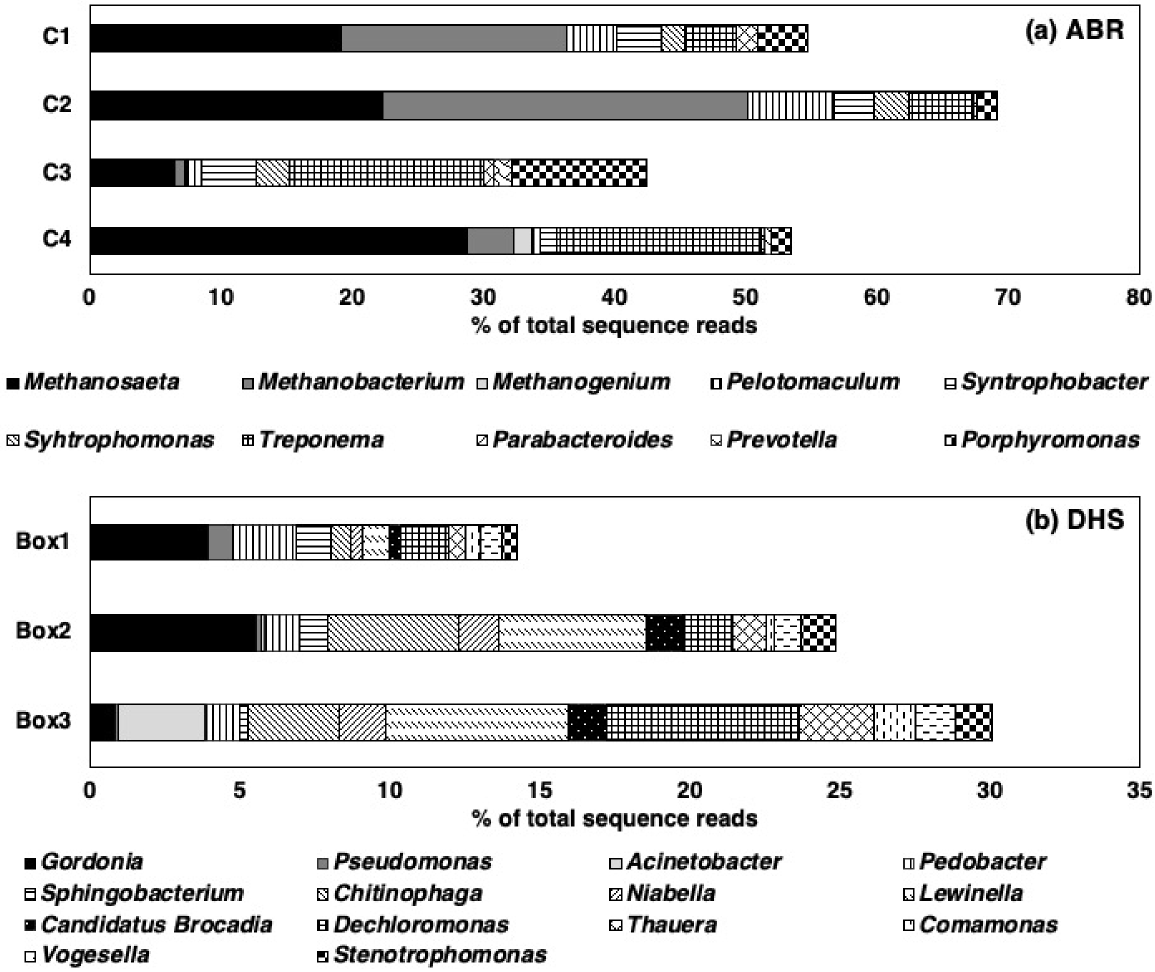

Fig. 6 *Ratios of the total sequence of the predominant microorganisms in: (a) ABR, and (b) DHS reactor on operating day 75*

The rubber-degrading bacterium *Gordonia* was predominantly detected in the first and second boxes of the DHS reactor. However, *Gordonia* was not detected in the PTC, even though it was highly contained in the seed core rubber. Rubber accumulation was confirmed at the surface of the sponge carriers in the first and second boxes. The ABR effluent flew along the surface of accumulated rubber with a large contact area of air in the DHS reactor. In contrast, core rubber was immersed in the wastewater in the PTC. These results indicated that core rubber acted as a biomass carrier for *Pseudomonas*, and *Pseudomonas* mainly degraded liquid rubber in the PTC. On the other hand, core rubber acted as a core substance for rubber coagulation, and *Gordonia* mainly degraded coagulated solid rubber in the DHS reactor. As nitrogen removers, anaerobic ammonia-oxidising (anammox) bacteria, such as *Candidatus Brocadia*, and denitrifying bacteria, such as *Dechloromonas*, *Thauera*, *Comamonas*, *Vogesella*, and *Stenotrophomonas*, were increased at the second and third boxes of the DHS reactor. Besides, *Dechloromonas* and *Thauera* were predominantly detected in the constructed wetland conducting solid-phase denitrification [27]. Furthermore, carbohydrate- and sugar-degrading bacteria, such as *Pedobacter*, *Chitinophaga*, *Niabella*, and *Lewinella*, were also increased at these boxes. Nitrifying bacteria, such as *Nitrobacter*, *Nitrosospira*, and *Nitrosovibrio*, were also detected with a detection ratio of less than 0.2%. Therefore, these microorganisms contributed to nitrogen removal by nitrification, anammox, and heterotrophic denitrification with sodium acetate and/or intermediates of residual rubber degradation in the DHS reactor.

Only *Pseudomonas* and *Acinetobacter* were highly detected in both the PTC and the DHS reactor. Some species of these bacteria were reported as heterotrophic nitrification-aerobic denitrification (HN-AD) bacteria [28], [29]. Additionally, solid carbon sources, which slowly release carbon, can enhance denitrification under low C/N ratio conditions [30]. In this study, however, nitrogen removal was confirmed only in the DHS reactor and not in the PTC because nitrification did not occur in the PTC. Thus, *Pseudomonas* and *Acinetobacter* primarily contributed to rubber removal and nitrogen removal in the PTC and DHS reactors, respectively. Furthermore, the accumulated rubber can serve as a solid carbon source for HN-AD bacteria, enhancing nitrogen removal in the DHS reactor.

4. Conclusion

At an early stage of the PTC, all particle sizes of residual rubber were enlarged to coagulation, whereas at a later stage of the PTC, all particle sizes of residual rubber degraded to low-molecular substances. Additionally, the biodegradability of NR wastewater was increased with longer HRT. These results indicated that the effect of HRT on coagulation and degradation of residual rubber was greater than that of particle properties. Also, trickling filter-type core rubbers had better performance for rubber coagulation than immersed-type core rubbers. Furthermore, the predominant rubber-degrading bacteria of trickling filter-type and immersed-type core rubbers were *Gordonia* and *Pseudomonas*, respectively. Thus, the core rubber in the trickling filter functioned as a core substance for rubber coagulation, and *Gordonia* degraded the accumulated solid rubber. In contrast, core rubber in the PTC functioned as a biomass carrier, and *Pseudomonas* degraded liquid rubber in the NR wastewater. In the DHS reactor, accumulated rubber retained rubber-degrading bacteria also functioned as a solid carbon source for denitrification. Therefore, the application of the PTC can contribute to rubber removal from NR wastewater, and the applicability of recovered rubber in the PTC to a solid carbon source for denitrification in the DHS reactor was considered.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***Study conception and design:*** *D. Tanikawa, Z. Kimura;* ***Data collection:*** *T. Ueno;* ***Analysis and interpretation of results:*** *D. Motokawa, Y. Itoiri, Y. Shinto;* ***Draft manuscript preparation:*** *D. Tanikawa, Z. Kimura. All authors reviewed the results and approved the final version of the manuscript.*

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