Investigate the effect of acetone on the anaerobic degradation process of latex wastewater

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1. Introduction

Natural rubber has been widely used for gloves, adhesives, and tires. The natural rubber latex consists of 35% cis-1,4-polyisoprene (or rubber hydrocarbon) and non-isoprene compounds, including 1–2% of proteins, 1–2% of acetone extract, 1.3% lipids, and other inorganic compounds [1]. Several proteins present in natural rubber latex cause latex allergy and, in some cases, provoke anaphylactic reactions [2]. Therefore, several research have been conducted on methods of protein removal from latex. Acetone is one of the candidate chemicals for removing protein compounds in rubber products [3]. Besides, the used acetone is thereby released with wastewater after latex processing. However, the existence of acetone in influent is assumed to harm the microorganism community in the anaerobic treatment of latex wastewater [4]. Therefore, this study aims to evaluate the effect of acetone on the anaerobic degradation process of latex wastewater.

2. Materials and methods

2.1. Batch experiment

A series of five glass vials with different ratios of acetone at 0%, 0.05%, 0.1%, 0.15%, and 0.25%; and latex at 0.144%, 0.11%, 0.084%, 0.056%, and 0%, respectively, was set up. The total volume of each vial was 730 mL, filled with 200 mL of anaerobic sludge and 450 mL of synthetic latex wastewater. Anaerobic sludge with mixed liquor suspended solids (MLSS) of 8700 ± 72 $mg \cdot L^{-1}$ and mixed liquor volatile suspended solids (MLVSS) of 6122 ± 63 $mg \cdot L^{-1}$ was collected from Nagaoka Chuo sewage treatment center (Nagaoka, Japan). Synthetic wastewater contained latex and acetone, equivalent to a COD concentration of 3000 $mgCOD \cdot L^{-1}$. Each vial was removed oxygen from the bottle headspace by the nitrogen gas for 2 min and was sealed with rubber stoppers immediately afterward. All vials were then incubated at 35°C in Bio-Shaker (BR-3000LF, Taitec) for 3 cycles of 7 days each.

2.2. Sampling and analytical methods

Wastewater sampling was conducted daily with measurements of chemical oxygen demand (COD) including tCOD and sCOD, total nitrogen (TN), and volatile fatty acids (VFAs). Biogas composition was determined daily using a GC-8A gas chromatograph equipped with a thermal conductivity detector. tCOD and sCOD were analyzed using a DR 2700™ portable spectrophotometer. TN was analyzed using HACH testing kits DR/10071 by DR 2700™ Portable Spectrophotometer. Sample for VFAs was prepared by using 0.22 μm filter and measured by using the Agilent 7100 Capillary Electrophoresis System.

2.3. DNA extraction, PCR amplification and 16 S rRNA gene sequencing

Retained sludge were collected at the end of the last cycle and stored at -20 °C for characterizing microorganisms. Genomic DNA was extracted from collected samples using the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) based on the manufacturer's protocol.

Retained sludge were collected at the end of the last cycle and stored at -20 °C for characterizing microorganisms. Genomic DNA was extracted from collected samples using the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) based on the manufacturer's protocol. Amplification of the bacterial 16S rRNA genes was conducted using a forward primer Univ515F (5′−GTGCCAGCMGCCGCGGTAA−3′) and a reverse primer Univ806R (5′−GGACTACHVGGGTWTCTAAT−3′). 16S rRNA gene sequencing was carried out using an iSeq 100 i1 reagent and an iSeq 100 system (Illumina Inc., USA).

2.4. Data analysis

As for microorganisms, sequence reads were analyzed using the QIIME 2 software [4]. Taxonomic classification was determined using the SILVA database.

3. Results and discussion

3.1. Batch experiment: COD removal performance

In the acetone-only reactor, tCOD concentration was maintained higher than 3000 mg.L^{-1}, with the fluctuation being recorded from 1% to 18% *(Fig.1)*. Meanwhile, in the latex-only reactor, 55 - 82% tCOD was removed from the influent *(Fig.1)*. The tCOD change indicated that while most of the latex was removed for the anaerobic degradation process, the majority of acetone was not degraded. Besides, after the anaerobic degradation process, even all the latex was treated, acetone was maintained at high COD values, thereby affecting the COD removal efficiency of latex wastewater. Based on the sCOD performance in all vials *(Fig.2)*, most of the soluble-acetone compound was kept in the same percentages with the influent, which can support the conclusion that acetone was not used for the anaerobic degradation in batch scales of latex wastewater.

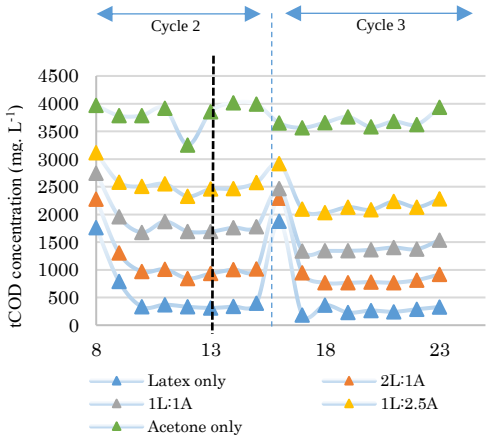

Fig.1: tCOD performance from day 8th to day 23th of batch experiments

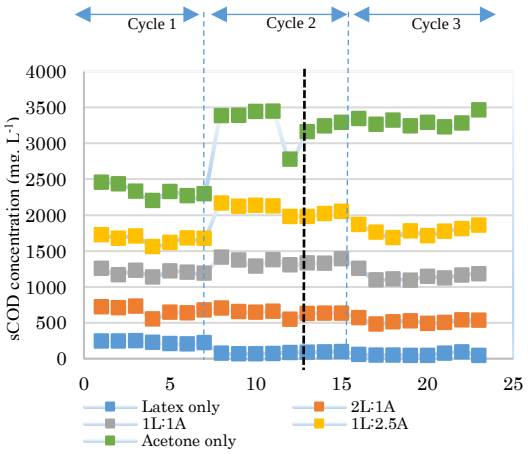

Fig.2: sCOD performance from day 1st to day 23rd of batch experiments

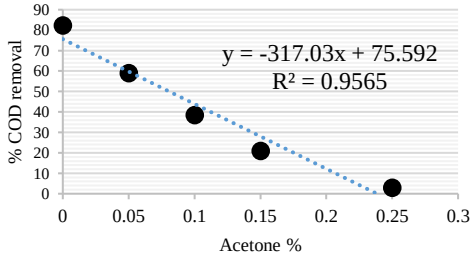

Fig.3: Standard curve for the effect of aceton on the processing efficiency of latex on the 13th day of batch experiment

Fig. 3 shows the standard curve for the effect of acetone on the processing efficiency of latex on the day 13 of the batch experiment. Until the day 13 of the batch experiment, the COD removal values became more stable (Fig.22. The correlation of acetone and COD removal in this standard curve indicated that the higher percentage of acetone, the lower percentage of COD removal in latex wastewater. Besides, the equation $y=-317.03x + 75.592$ (*) can be applied to investigate the correlation between the percentage of acetone and the percentage of COD removal in latex wastewater, giving initial assessment of latex treatment efficiency under the influence of acetone.

3.2. Biodiversity and distribution of microorganism in the batch scales

As shown in Fig.6, *Thauera* sp. was the most dominant bacteria, with a detection rate of 27.76% in the acetone-only reactor. Moreover, in other reactors, the more percentage of acetone, the more *Thauera* was detected. *Thauera* can use acetone as the energy source for its denitrification process with nitrate and nitrite as a carbon source [5]. *Curvibacter* and *Phenylobacterium* were mostly detected in the reactor of only latex, with detection rates of 5.87% and 3.75%, respectively. *Curvibacter* can use nitrate and nitrite as carbon and energy sources [6], while *Phenylobacterium* can use organic acid, fatty acid, and amino acid from the latex degradation process as the carbon source [7]. *Phenylobacterium* can also join in the denitrification process [7]. These bacteria may have been involved in latex degradation process. *Brachymonas denitrificans* was abundantly

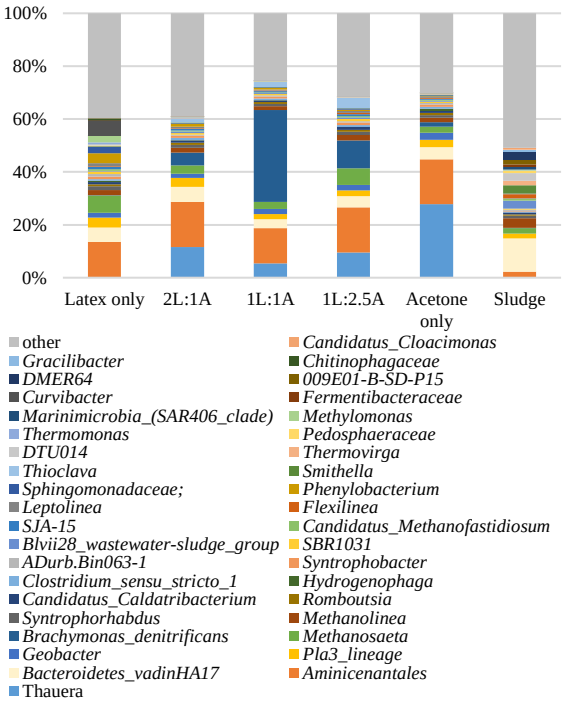

Fig.4: Microorganism community at genus

identified in [1A:1L] reactor, with detection rate of 34.83%. *Brachymonas* can use organic acids and some amino acids as carbon source and nitrate as energy source for the denitrification process [8]. In addition, *Aminicenantales* was predominated in all the vials, with detection rate from 13.34 to 17.16% despite only 2.3% of *Aminicenantales* detected in the sludge sample. *Aminicenantales* is reported to have nitrite reduction ability [9]. From the preferred existence of these bateria, it can be concluded that these bacteria may use the products from both latex and acetone degradable bacteria for their own degradation. One hypothesis has been given to a symbiotic process among many groups of bacteria that use acetone, latex, and nitrate compounds for their anaerobic process.

4. Conclusion

This study conducted a batch experiment simulating latex wastewater at different acetone

concentrations to evaluate the effect of acetone on the anaerobic reduction of latex. The results showed that the higher the concentration of acetone, the lower the COD removal efficiency. This linear relationship could be expressed by the equation $y = -317.03x + 75,592$, $R^2 = 0.9565$. In addition, the results showed that a large amount of acetone was not degraded during the running phases, indicating the potential for acetone extraction during latex treatment. The most prominent genera, such as *Thauera*, *Curvibacter*, *Phenylobacterium*, *Brachymonas* and *Aminicenantales*, involved in latex and acetone degradation and denitrification, were identified in vials.

Acknowledgment

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