Fate and Effect of PET Microplastics in Anaerobic Sludge Digestion After Thermal and Pancreatin Enzyme Combined Disintegration

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Introduction

Microplastics (MPs) in wastewater treatment plants (WWTPs) have become an increasing concern due to their persistence and accumulation. More than 90% of MPs entering WWTPs are retained in waste-activated sludge (WAS). However, few studies have investigated the effects of MP abundance on the anaerobic digestion (AD) process of WAS. Existing research suggests that MPs hinder digestion by reducing methane production and causing higher retention of organic matter and nutrients (Li et al., 2020). Meanwhile, due to the complex floc structure and strong cell membranes of sludge, the hydrolysis step in AD becomes rate-limiting. To overcome this limitation and enhance stabilization efficiency, various disintegration processes have been applied to sludge. These methods primarily aim to solubilize sludge solids through thermal, mechanical, chemical, biological, or combined techniques. By accelerating hydrolysis, these approaches not only maximize biogas production in anaerobic digesters but also minimize the volume of sludge requiring disposal (Volschan Junior et al., 2020). Therefore, this study aims to investigate the fate of polyethylene terephthalate (PET) MPs in AD of sludge subsequent to thermal and enzymatic combined disintegration while also examining the effects of PET MPs on the AD process. This study is the first to investigate the use of pancreatin enzyme for the disintegration of WAS, an enzyme that has the potential to deteriorate PET MPs due to their susceptibility to hydrolytic enzyme attack (Müller et al., 2005). It was selected due to its unique composition of five enzymes (trypsin, amylase, lipase, ribonuclease, and protease) which target various bonds within both WAS and MPs (Rana, 2019). Additionally, the combination of thermal and enzymatic disintegration has not been applied before in literature and remains as an unexplored area is examined in this study. To enhance methane yield in AD, this novel approach aims to accelerate hydrolysis and maximize methane production while potentially breaking down PET MPs accumulated in sludge.

Materials and Methods

WAS and anaerobically digested seed (ADS) were sampled from the Central WWTP in Ankara and used at a total solids (TS) concentration of 2%. The pancreatin enzyme (P7545), obtained from Sigma-Aldrich, was used in this study. PET MPs were prepared by cutting water bottles and passing them through 250-500 µm sieves.

The thermal disintegration process was applied to the sludge samples (WAS) containing MPs using an autoclave at 127ºC for two hours. After optimization studies, pancreatin enzyme used at 150 mg/g TS dose for 24 hours at 38ºC and 100 rpm following thermal disintegration. Disintegration was monitored by measuring the reduction in volatile suspended solids (VSS) and the increase in the degree of disintegration (DD).

Biochemical methane potential (BMP) tests were conducted with disintegrated (ME) and control (MEC) sludge samples, each containing varying doses of MPs (0, 1, 3, 6, and 10 mg/g TS) under mesophilic conditions (35ºC) in triplicate 250 mL serum bottles. Seed control (SC) and enzyme control (EC) reactors were set up to observe seed activity and eliminate methane produced from the enzyme's organic structure. Abiotic conditions were tested with an inhibited seed to assess the fate of PET in abiotic conditions and observe the impact of biological activity. Cumulative biogas production was measured using the water displacement method, while methane content was analyzed by gas chromatography with thermal conductivity detection (GC-TCD). The physicochemical and morphological properties of PET MPs before and after disintegration and digestion were analyzed using Fourier transform infrared (FTIR), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM).

Results and Discussion

Disintegration Efficiency and Reactor Performance

After combined thermal and enzymatic disintegration, the disintegrated reactors showed a DD of 51.5%, while the control reactors showed only 11.6% DD. The reduction in VSS was consistent with the DD results, with the disintegrated reactors showing a 33.8% VSS reduction, compared to only 10.2% in the controls. Additionally, the soluble protein content of WAS increased by 52.6 times, and the soluble carbohydrate content increased by 33.2 times in disintegrated reactors. As a result of digestion, the methane production efficiency of the reactors subjected to disintegration is 17.6% higher compared to the controls. Overall, reactor performance parameters, including TS, VS, and tCOD removal rates, were enhanced in the disintegrated reactors. However, no statistically significant correlation was observed between the reactors based on the MP dose, in terms of both net methane yield and reactor performance ($p>0.05$). Methane production was not observed in the abiotic reactors.

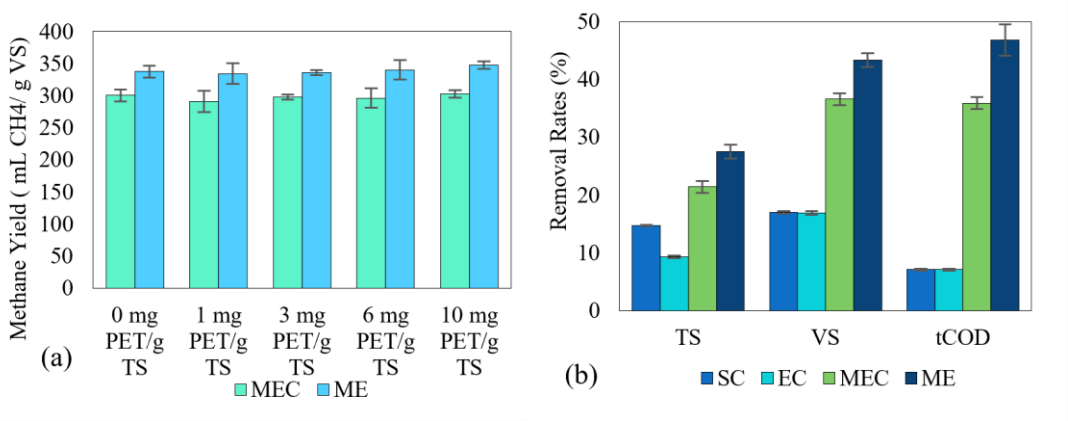

Figure 1. (a) Net methane yields and (b) removal rates of the reactors

Physicochemical and Morphological Changes of PET

Significant changes in the morphology of PET microplastics were observed during the disintegration and digestion processes. During disintegration, the surface roughness of the microplastics increased, and deep peeling occurred at the edges, which enhanced damage on the surface, making them more susceptible to biological processes. During digestion, more indentations, protrusions, and wear were observed on the surface of PET MPs in the biotic reactors, indicating that biological activity had a greater effect on morphological changes. In abiotic conditions, surface changes were less pronounced, suggesting that biological processes contributed more to altering the morphological structure.

The physicochemical changes, including the Carbonyl Index (CI) and crystallinity alterations, were assessed. Following disintegration, the CI value showed a decrease of approximately 4.8%; however, no statistically significant differences were observed between the CI values across reactors with different MP doses. Additionally, disintegration did not have a significant impact on crystallinity ($p>0.05$). After digestion, no notable changes in the crystallinity of PET MPs were observed under both biotic and abiotic conditions, and no differences were detected compared to the initial stage. These findings suggest that neither disintegration nor digestion influenced the crystallinity structure of PET microplastics, and that microorganisms or other biological activities did not alter this structure. Further details, including SEM images, CI values, and crystallinity results, will be provided and thoroughly discussed in upcoming presentations.

Conclusions

This study demonstrated that combining thermal and enzymatic disintegration improved the efficiency of anaerobic digestion of WAS. The disintegration process resulted in a significant reduction in VSS and a 51.5% increase in DD. Methane production improved significantly by 17.6% compared to control reactors. Contrary to existing literature, PET MPs did not influence methane production, regardless of the dose applied. The morphological changes observed in the MPs indicated that biological processes had a stronger impact on their surface structure, with combined disintegration playing a key role in these alterations. However, both disintegration and digestion did not significantly affect the crystallinity structure or CI of the PET MPs. Overall, this approach holds promise for enhancing methane production in anaerobic digestion but demonstrates a limiting effect on the accumulated PET MPs in the sludge.

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