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Effect of microplastic on anaerobic digestion of wasted activated sludge

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HIGHLIGHTS

- Microplastics inhibited methane production in anaerobic digestion.
- Methane production potential and hydrolysis coefficient decreased with the existence of microplastics.
- Methane production potential inhibition could attribute to incomplete digestion with existence of microplastics.
- Microbial community shows no significant difference with and without microplastics.

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ABSTRACT

Over 90% of microplastics that enter wastewater treatment plants end in the wasted activated sludge. The effect of microplastic abundance on the activated sludge anaerobic digestion has been rarely reported. This study investigated the methane production performance during anaerobic digestion with different abundance of microplastic doses (0, 1,000, 3,000, 6,000, 10,000, 30,000, 60,000, 100,000 and 200,000 polyester particle/kg activated sludge). The methane production was reduced to 88.53 ± 0.5%, 90.09 ± 1.2%, 89.95 ± 4.7%, 95.08 ± 0.5%, 90.29 ± 0.5%, 93.16 ± 0.8%, 92.92 ± 1.3%, and 92.72 ± 0.6% as compared with control after digestion for 59 days. The methane production of all conditions was fitted with the logarithm model ($R^2 > 0.95$) and one-substrate model ($R^2 > 0.99$). The predicted and actual methane production values of digestion for 59 days had high correlation in all conditions with $R^2 > 0.95$. The analysis based on the biochemical methane potential test model indicated that the methane production potential (B_0) and hydrolysis coefficient (k) decreased at nearly all tested conditions. The reactor digestate with microplastics retained higher organic matter and nutrient concentration and had slightly lower dewaterability than the control. The inhibition of methane production potential could be attributed to the incomplete digestion with the existence of microplastics. The microbial community showed no significant difference with and without microplastics.

1. Introduction

Municipal wastewater treatment plants (WWTPs) are receptors of microplastics from many sources of human activities (Mason et al., 2016; Kang et al., 2018). The human activities including the use and discard of personal care products and other plastic pieces, synthetic clothing such as polyester (PES) and nylon could also shed thousands of fibers into the wastewater during washing process, those could finally enter the WWTPs through urban sewer system (Li et al., 2019; Sun et al., 2019; Cheung and Fok, 2017; Napper and Thompson, 2016). Although the WWTPs effluent still contains microplastics, over 90% of microplastics that enter the WWTPs are finally removed and retained in the sludge (Carr et al., 2016; Ziajahromi et al., 2016; Karapanagioti, 2017; Raju et al., 2018). Lares et al. (2018) conducted microplastics removal by conventional activated sludge process and membrane bioreactor and found that the retention capacity of microplastics in the studied WWTPs is 98.3%. Sun et al. (2019) reviewed the occurrence and fate of microplastics in WWTPs and concluded that, above 88% of microplastics are removed in WWTPs without tertiary treatment and over 97% are removed with tertiary treatment. Considering the increasing in plastic production and use, the microplastic

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abundance in the excess sludge can also increase yearly. Among the techniques used for sludge treatment, anaerobic digestion is the most promising and common sewage sludge treatment process. It can remove odors and pathogens, stabilize sludge, reduce sludge volume, and produce renewable energy. Anaerobic digestion can reduce the capital operation cost of WWTPs due to the methane production and is regarded as an essential part of modern WWTPs (Mao et al., 2015; Zhen et al., 2017; Wang et al., 2017; Ma et al., 2018; Vasco-Correa et al., 2018).

The most frequently detected microplastics from WWTPs influent and effluent are polyester (PES, approximately 28%–89%), polyethylene (PE, nearly 4%–51%), polyethylen terephthalat (PET, around 4%–35%), and polyamide (PA, approximately 3%–30%) (Sun et al., 2019). The most frequently used dimensions for size classification of microplastics in WWTPs are 25, 100, and 500 μm. Over 70% of which found in the influent of WWTPs are over 500 μm, and over 90% found in the effluent are smaller than 500 μm. A high percentage of microplastics detected in WWTPs has a size at 10–500 μm. Sun et al. (2019) concluded that microplastics with a small size in the WWTPs should be investigated. Apart from the difference in the size of microplastics in the WWTPs, the abundance of microplastics in sewage sludge also varies in different countries. The abundances can be varied from 50 particles/kg sludge to 170,900 particles/kg sludge (Carr et al., 2016; Murphy et al., 2016; Leslie et al., 2017; Lares et al., 2018; Li et al., 2018; Mahon et al., 2016; Mintenig et al., 2017). Removing microplastics from the wasted sludge is difficult (Talvitie et al., 2017), and the most commonly used method for microplastic detection from the sludge is gravity flotation. High-dense solutions, such as zinc chloride ($ZnCl_2$, 1.5 g/cm^3), are widely used to wash out a wide density range of microplastics (Imhof et al., 2013; Lee and Kim., 2018; Sun et al., 2019). The microplastics retained in sewage sludge of WWTPs has a wide range of types, sizes, and shapes. This condition causes difficulty in removing microplastics from the sludge.

Wei et al. (2019b) reported that the existence of microplastics polyethylene terephthalate (PET) inhibited the sludge hydrolysis, acidogenesis, and acetogenesis processes and thus inhibited the hydrogen production in alkaline anaerobic fermentation of wasted sludge. The existence of microplastic PET has posed the microbial structure change toward hydrolysis-acidification. Microplastics PET can release toxic di-*n*-butyl phthalate (DBP) and reactive oxygen species (ROS) during the alkaline anaerobic digestion process and can thus negatively affect the process. Wei et al. (2019)a also found that the polyvinyl chloride (PVC) can leach toxic Bisphenol-A and can thus inhibit the anaerobic digestion process. Fu et al. (2018) investigated the effects of polystyrene nanoparticle (average size of 54.8 nm) in anaerobic digestion systems. The nanoparticles can attach on the surface of cell membrane and inhibit the growth and metabolism of some of the microbes. Therefore, the different sizes, abundances, and materials of microplastics have different effects on the activated sludge anaerobic digestion process. The anaerobic digestion operation condition can also affect the leaching of microplastic toxic compounds and thus affect the digestion process. Further investigation on the effects of microplastics in sludge on the anaerobic digestion process is important.

This study aims to investigate the effect of the most frequently detected microplastic polyester (PES) at a wide range of microplastics abundances on the methane production during the conventional waste activated sludge (WAS) anaerobic digestion process. The accumulative methane production under varying microplastic abundances was investigated and compared by biochemical methane potential (BMP) tests. The methane production potential and hydrolysis rate of the WAS were discussed by model-based analysis. The effects of microplastic addition on the changes in microbial community structure before and after digestion under various conditions were also investigated.

2. Materials and methods

2.1. Sludge sources and added microplastic abundance

The inoculum and secondary sludge used were collected from anaerobic digester and secondary sedimentation tank of two different municipal WWTPs in Wuhan City, China, respectively. The secondary sludge was used as substrate, and the inoculum was used to biodegrade the secondary sludge. Table 1 lists the properties of the inoculum and secondary sludge. Batch experiments were conducted with different microplastic abundance of 0, 1,000, 3,000, 6,000, 10,000, 30,000, 60,000, 100,000 and 200,000 particles/kg total solids (TS), as displayed in Table 2. The added microplastics had a size of 200 μm (Shanghai Youngling Electromechanical

Table 1
Primary properties of secondary sludge and inoculum (mean ± 95% confidence interval from triplicate measurements).

Parameter	Secondary sludge	Inoculum
Total Solids (TS) (g/L)	15.6 ± 0.4	28.4 ± 0.4
Volatile Solids (VS) (g/L)	9.5 ± 0.5	12.5 ± 0.5
Total Chemical Oxygen Demand (TCOD) (g/L)	26.2 ± 0.3	7.96 ± 0.12
Soluble Chemical Oxygen Demand (SCOD) (g/L)	0.5 ± 0.1	5.2 ± 0.96
pH	7.18 ± 0.2	7.35 ± 0.4

Table 2
Experimental design with different abundance of microplastics PES added.

Reactor	Function	Experimental conditions
R0	Blank	105 mL inoculum + 70 mL MilliQ water
R1	Control	105 mL inoculum + 70 mL 2nd sludge
R2	MP1	105 mL inoculum + 70 mL 2nd sludge at MP 1000 particle/kg·TS
R3	MP2	105 mL inoculum + 70 mL 2nd sludge at MP 3000 particle/kg·TS
R4	MP3	105 mL inoculum + 70 mL 2nd sludge at MP 6000 particle/kg·TS
R5	MP4	105 mL inoculum + 70 mL 2nd sludge at MP 10,000 particle/kg·TS
R6	MP5	105 mL inoculum + 70 mL 2nd sludge at MP 30,000 particle/kg·TS
R7	MP6	105 mL inoculum + 70 mL 2nd sludge at MP 60,000 particle/kg·TS
R8	MP7	105 mL inoculum + 70 mL 2nd sludge at MP 100,000 particle/kg·TS
R9	MP8	105 mL inoculum + 70 mL 2nd sludge at MP 200,000 particle/kg·TS

Technology, China). The corresponding amount of microplastics was added into each reactor before adding sludge.

2.2. Biochemical methane potential (BMP) tests

BMP tests were conducted to assay the methane production by secondary sludge with the different abundances of microplastics. After adding of microplastics into the reactor, the inoculum and secondary sludge were added in reactor with a volatile solids (VS) ratio of 2:1. A total of 105 mL inoculum and 70 mL secondary sludge were added into a 310 mL reactor and totally mixed. Pure N_2 gas was used to flush the sludge for 5 min to create anaerobic condition inside the reactor. Then, a rubber stopper was used to seal the reactor. The blank test containing the same inoculum of 105 mL and 70 mL MilliQ water instead of secondary sludge was also conducted. The pH values were recorded as 7.0 ± 0.2, and the temperature was controlled at 36 ± 1 °C. The batch tests were conducted in triplicate in a constant temperature shaking table for 59 days until the biogas production decreased to negligible. The biogas methane production was recorded daily in the first 7 and 2–4 days thereafter. The methane volume was calculated by multiplying the methane percentage in the biogas and its concentration. The methane production by the secondary sludge in each reactor was determined by subtracting the methane produced from the blank test and recorded as methane volume per VS mass in unit $L{\cdot}CH_4/kg{\cdot}VS$.

2.3. Model-based analysis of BMP test results

One-substrate model, as shown in Equation (1), was used in this study. The two key parameters associated with methane production, namely, hydrolysis rate (k) and biochemical methane potential (B_0), were used to evaluate and compare methane production kinetics and the potential of the WAS added with different abundances of microplastics. B(t) ($L{\cdot}CH_4/kg{\cdot}VS$) is the biochemical methane production at given time t (d).

$$B(t) = B_0 * (1 - e^{-kt}) \quad (1)$$

The degradation extent (Y, %) of sludge was evaluated by using B_0 with Equation (2). In Equation (2), B_0 is the biochemical methane potential in this study, and 380 is the secondary sludge theoretical biochemical methane potential at standard conditions (1 atm, 25 °C). Rss is the measured volatile solids/total chemical oxygen demand (TCOD) of the secondary sludge (i.e., 0.36 in this study).

$$Y = B_0/380_{*Rss} \quad (2)$$

Logarithm model, as shown in Equation (3), was also used in this study to simulate the methane production. In the equation, Yt ($L{\cdot}CH_4/kg{\cdot}VS$) is the cumulative methane production in day t.

$$Yt = a*\ln(t) + b \quad (3)$$

2.4. Analytical methods

The TS, VS, TCOD, and soluble chemical oxygen demand (SCOD) concentration of the sludge were measured before batch test. The dissolved samples were measured before filtering with 0.45 μm millipore filter. The SCOD, carbohydrate, NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}, and fluorescence excitation emission matrix (FEEM) of the digested sludge supernatant after the digestion were also tested. All the parameters were measured in triplicate following the standard methods (APHA, 2012). Bulk NH_4^+-N, NO_2^--N, and NO_3^--N concentrations were analyzed spectrophotometrically (UV-6100, Mapada, China). FEEM was analyzed by a cryogenic fluorescence spectrometer with the emission wavelength of 280–540 nm and the excitation wavelength 200–400 nm (QM-4CW, PTI, USA). The dewatering rate of the sludge was determined by measuring the free water volume of samples after centrifugation at 4000 rpm for 10 min. The pressure in the reactors were measured by manometer before sampling. The volume of the biogas generated was calculated on the basis of the pressure increase in the BMP test reactor headspace. The biogas composition and methane concentration were measured by a gas chromatographic (GC) analyzer (GC7890, Agilent, USA).

2.5. Microbial community analysis

The microbial community structure of the sludge in the control reactor (R1) before and after digestion and reactors with microplastic abundances of 10,000 and 200,000 particle/kg TS (R5 and R9) after digestion were investigated. The sludge samples were collected and centrifuged for 5 min at 10,000 rpm. The DNA of the samples was extracted using DNA extraction kit. Concentration and purity were measured using the NanoDrop One (Thermo Fisher Scientific, MA, USA). The primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTA CHVGGGTWTCTAAT-3′) targeting the V4 region of bacteria and archaeal 16S DNA genes were used for PCR test (primer, Invitrogen, USA; PCR, BioRad S1000, USA). The samples were then sequenced using Illumina Hiseq 2500 (PE250) platform. The operation taxonomic units (OTUs) used the 97% identity thresholds for the taxonomic classification analysis (Wang et al., 2017; Wei et al., 2019a). The Chaos index indicate the richness of species in the sample. The Shannon index and PD whole tress index indicate the richness and uniformity of species, under the same richness, higher uniformity indicates a higher species diversity.

2.6. Statistical analysis

Analysis of variance was used to evaluate the significance of results, where $p < 0.05$ means statistically significant and $p > 0.05$ was considered as statistically insignificant. The linear correlation between the measured and predicted BMP value was analyzed by logarithm model and one-substrate model.

3. Results and discussion

3.1. Effect of microplastic abundance on the biochemical methane production

The cumulative methane production from secondary sludge under different abundances of microplastics added was show in Fig. 1. The measured methane production value and simulated methane production curves by the logarithm model fit after digestion for 59 days were also displayed. The control represents the sludge that was used for anaerobic digestion without adding microplastics, and the methane production of the control and tests with different concentration of microplastic added was calculated by subtracting the methane production from the blank sample. The BMP test with PES at different abundances was conducted for 59 days, where the complete anaerobic digestion was achieved and the methane production reached a stable level. The cumulative methane production values after 59 days of anaerobic digestion at microplastic abundances of 0, 1,000, 3,000, 6,000, 10,000, 30,000, 60,000, 100,000, 200,000 particles/kg·TS were 233.5 ± 1.0, 206.7 ± 0.5, 210.4 ± 1.2, 210.0 ± 4.7, 222.0 ± 0.5, 210.8 ± 0.5,

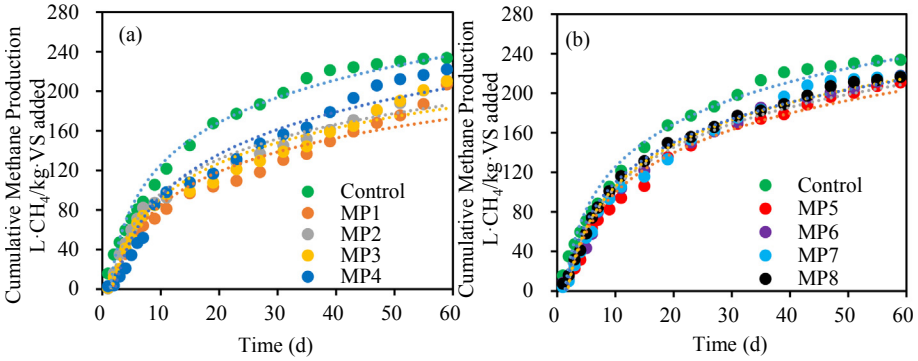

Fig. 1. Cumulative methane production from secondary sludge under different MP added, Measured and simulated value curves by logarithm model fit. (Symbols represent experimental measurements and lines represent model fit.)

217.5 ± 0.8, 217 ± 1.3 and 216.5 ± 0.6 L·CH_4/kg·VS, respectively (mean ± 95% confidence interval). The methane production in reactors with microplastics existed were lower than that of the control in all cases tested. The control reactor had high methane production at the first 7 days and the day 59th (Fig. 1). The methane production between different abundances of microplastics added did not show considerable difference, as displayed in Fig. 1. The addition of microplastics have inhibited the methane production to 88.53%, 90.09%, 89.95%, 95.08%, 90.29%, 93.16%, 92.92%, and 92.72% of the control. The addition of PES inhibited the methane production at approximately 10%. The Pearson correlation between the microplastic abundance and the accumulative methane production was 0.026. This finding implied that the microplastics PES abundances had slight correlation with the methane production. The measured and predicted methane production values showed a linear correlation with $R^2 > 0.95$ in all the tested conditions (Fig. 2a). Thus, the logarithm model could be used to simulate the methane production in each reactor with microplastics. Fu et al. (2018) found that the methane yield and maximum daily methane yield was decreased for 14.4% and 40.7% at nanoplastic concentration of 0.2 g/L. Wei et al. (2019)a reported that microplastic polyvinyl chloride at 20, 40, and 60 particles/g·TS has inhibited methane production to 90.6 ± 0.3%, 80.5 ± 0.1%, and 75.8 ± 0.2% of the control. The methane production inhibition in this study was not as high as earlier publications. This implied that the anaerobic methane production inhibition by microplastics could be related with the size and characteristics of microplastics used.

3.2. Estimation of hydrolysis rate and BMP

The methane production potential and the hydrolysis rate during the anaerobic digestion were simulated by one-substrate model. The corresponding predicted methane production values after digestion for 59 days were 236.6 ± 2.4, 204.0 ± 12.8, 196.2 ± 8.6, 205.8 ± 11.9, 255.3 ± 13.2, 218.7 ± 4.4, 220.5 ± 4.3, 230.3 ± 5.2, and 214.7 ± 4.0 L·CH_4/kg·VS for microplastic abundance at 0, 1,000, 3,000, 6,000, 10,000, 30,000, 60,000, 100,000, and 200,000 particles/kg·TS, respectively ($p < 0.0001$). The corresponding hydrolysis rates were 0.065 ± 0.002, 0.038 ± 0.005, 0.054 ± 0.006, 0.044 ± 0.006, 0.033 ± 0.003, 0.049 ± 0.002, 0.052 ± 0.003, 0.049 ± 0.003, and 0.062 ± 0.003 d^{-1} ($p < 0.0001$, Table 3). The one-substrate model fitted well with the methane production results. Table 3 shows the BMP (B_0) and the hydrolysis rate (k) of all reactors ($R^2 > 0.97$, $p < 0.0001$ in all cases). Fig. 2b presents the actual value and predicted values of accumulative methane production based on one-substrate model. The measured and predicted values of methane production showed a linear correlation with $R^2 > 0.99$ (Fig. 2b). As shown in Table 3, the methane potential in the reactors with microplastic PES were all lower than those in the control. Although the microplastics added in 10,000 particles/kg·TS showed higher (107.92%) methane potential by prediction compared with the control, its actual methane production value was lower (95.08%) than that of the control. The secondary sludge degradation extent (Y) had a relatively higher value with microplastics added at 10,000 particles/kg·TS and had lower value in all other tests than in the control. Meantime, the hydrolysis rate of microplastics in 10,000 particles/kg·TS was the lowest

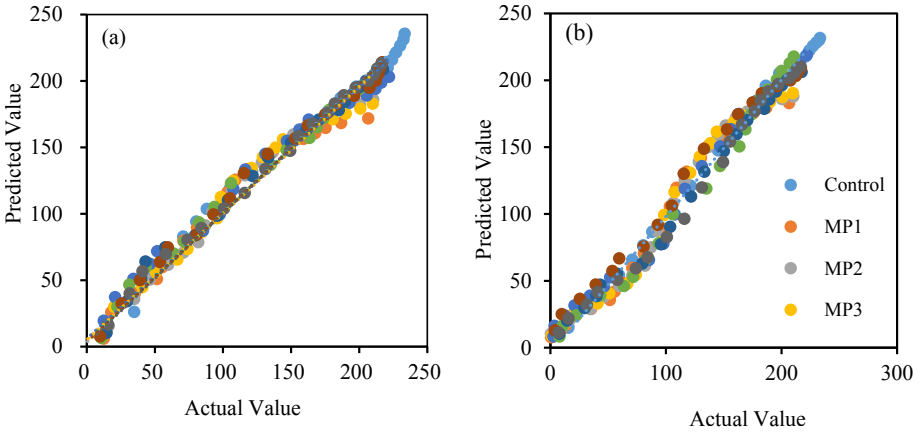

Fig. 2. Measured and Predicted biochemical methane production by logarithm model ((a), $R^2 > 0.95$) and one substrate model ((b), $R^2 > 0.99$).

Table 3
Determined hydrolysis rate (k), biochemical methane potential (B_0) of sludge at different microplastics abundance using one-substrate model.

MP added (particles/kg·TS)	0	1000	3000	6000	10,000	30,000	60,000	100,000	200,000
K (d^{-1})	0.065 ± 0.002	0.038 ± 0.005	0.054 ± 0.006	0.044 ± 0.006	0.033 ± 0.003	0.049 ± 0.002	0.052 ± 0.003	0.049 ± 0.003	0.062± 0.003
B_0 ($L{\cdot}CH_4/kg{\cdot}VS$)	236.6 ± 2.4	204.0 ± 12.8	196.2 ± 8.6	205.8 ±11.9	255.3 ± 13.2	218.7 ± 4.4	220.5 ± 4.3	230.3 ±5.2	214.7 ±4
R^2	0.9970	0.9828	0.9802	0.9791	0.9929	0.9970	0.9966	0.9961	0.9954
Y	0.224 ±0.002	0.193 ±0.012	0.186 ±0.008	0.195 ±0.011	0.242 ±0.013	0.207 ±0.004	0.209 ±0.004	0.218 ±0.005	0.203 ±0.004

($0.033 \pm 0.003\ d^{-1}$) compared with those of other tested conditions. In general, the hydrolysis rate of the tests with microplastics was lower than that of the control. Therefore, the addition of microplastics inhibited the hydrolysis rate and the accumulative methane production (Wei et al., 2019a,b; Fu et al., 2018). However, no correlation existed between the added microplastics abundances with the hydrolysis rate and the predicted methane production. This finding indicated that the methane production inhibition by the microplastics PES had no specific regulation.

3.3. Effects of microplastic abundance on digested sludge character

The methane production in anaerobic digestion process can be improved by pretreatment with advanced oxidation processes, in which the sludge destruction and extracellular polymer substrate releasement can be increased (Wang et al., 2013; Pijuan et al., 2012; Song et al., 2016). The anaerobic digestion process can also be inhibited with the existence of toxic compounds, such as heavy metals, ammonia, sulfide, organics and so forth, which negatively affect the microbial process or microbial activities (Altaş, 2009; Xu et al., 2017; Chen et al., 2008, 2014). The microplastic PES or other common microplastics in municipal WWTPs are generally inert and insignificantly affect anaerobic digestion (Cole et al., 2011; Eerkes-Medrano et al., 2015). They do not affect the sludge character change in short time. Its effects on the sludge character after anaerobic digestion for a long time is still unclear yet. These considerations could be useful in the analyzing of methane production potential in this study.

Table 4 shows the sludge characters including carbohydrate, NH_4^+-N, NO_3^--N, NO_2^--N, and PO_4^{3-}. The digestate after 59 days anaerobic digestion with microplastics added had higher carbohydrate, NH_4^+-N, and NO_3^--N concentrations than the control in nearly all the conditions tested. The corresponding free ammonia (FA), which could inhibit the microbial process, was calculated following the Equation reported by Anthonisen et al. (1976). The FA concentrations were 5.73, 6.82, 7.40, 7.33, 7.04, 7.42, 7.04, 7.17, 7.44, and 7.02 mg NH_3–N/L for the digestate in reactors R0-R9, respectively. These values were considerably lower than the effective concentration. Thus, the high ammonium detected in the reactors with microplastics was not the reason for methane production inhibition (Wang et al., 2018; Xu et al., 2018; Liu et al., 2018). The digestate in R2-R9 (with different abundances of added microplastics) still retained similarly higher concentrations of SCOD, carbohydrate, NO_3^--N and PO_4^{3-} than that in the control reactor. On the contrary, the UV_{254} value of the digestate in R2-R9 was lower than that in the control. This finding indicated that the digestate in R2-R9 retained high concentration of organic matters, nitrogen, and phosphorus. The recalcitrant components, that is, the UV_{254} value, in R2-R9 were lower than those in the control. This finding was also in accordance with the FEEM results as shown in Fig. 3. Therefore, the digestate sludge retained very high amount of fulvic acid-like recalcitrant components. The FEEM of digestate supernatant from R0 to R9 was tested. Fig. 3 shows only R2 and R5 because the results for R2-R8 were similar. As shown in Fig. 3, the digestate of R2 and R5 (with microplastics) had evidently lower intensity of fulvic acid-like components than that of the control R0 (Fig. 3b and c). Meanwhile, the digestate of R9 (with the highest microplastic abundance) had the lowest fulvic acid-like recalcitrant compared with those of the other cases. This finding indicated high digestion ability or high reduction in fluorescence response components in R9 (Li et al., 2014; Luo et al., 2013). The low fluorescence intensity in R9 could be attributed to the reduction in fluorescence response components and not methane potential (B_0) or hydrolysis rate (k). Thus, the different abundances of added microplastics led to relatively high amounts of retained organic matters and nutrients, which caused difficulty in anaerobic digestate treatment. This condition could be attributed to the incomplete digestion with the existence of microplastics. The sludge dewatering rate of digestate was also tested, as shown in Fig. 4. The dewatering rate of R2-R9 was slightly higher than that of the control. This result implied that the digestate dewatering ability was improved with the existence of microplastics.

Table 4
The digestate basic characters.

Reactor	Function	Carbohydrate (mg/L)		NH_4–N (mg/L)		NO_3–N (mg/L)		NO_2–N (mg/L)		PO_4^-(mg/L)		SCOD (mg/L)		UV_{254} (mg/L)*5	
		Aver.	SD	Aver.	SD	Aver.	SD	Aver.	SD	Aver.	SD	Aver.	SD	Aver.	SD
R0	Blank	15.98	15.98	394.17	1.75	0.46	0.10	0.01	0.01	38.19	0.48	272	0	0.240	0.003
R1	Control	18.84	2.85	468.59	4.43	0.55	0.03	0.01	0.00	37.83	0.84	476	0	0.329	0.012
R2	MP1	24.00	2.57	509.03	3.49	0.52	0.02	0.01	0.00	36.07	6.03	561	51	0.275	0.005
R3	MP2	27.40	2.28	503.65	2.69	0.73	0.11	0.04	0.03	33.03	3.56	918	34	0.280	0.004
R4	MP3	31.96	15.98	484.04	3.49	0.62	0.08	0.01	0.00	34.19	3.68	561	34	0.280	0.005
R5	MP4	37.10	24.54	510.10	5.10	0.60	0.00	0.01	0.00	33.47	2.32	1122	346	0.293	0.003
R6	MP5	29.11	10.84	484.31	2.15	0.97	0.04	0.01	0.01	31.15	3.52	742	198	0.285	0.009
R7	MP6	47.95	17.12	493.31	6.05	0.50	0.01	0.01	0.00	34.35	3.44	595	40	0.278	0.002
R8	MP7	37.67	11.42	511.31	11.15	0.55	0.03	0.02	0.01	40.35	1.60	436	0	0.283	0.001
R9	MP8	30.25	5.14	495.19	0.40	0.62	0.00	0.01	0.00	35.83	4.92	675	119	0.282	0.002

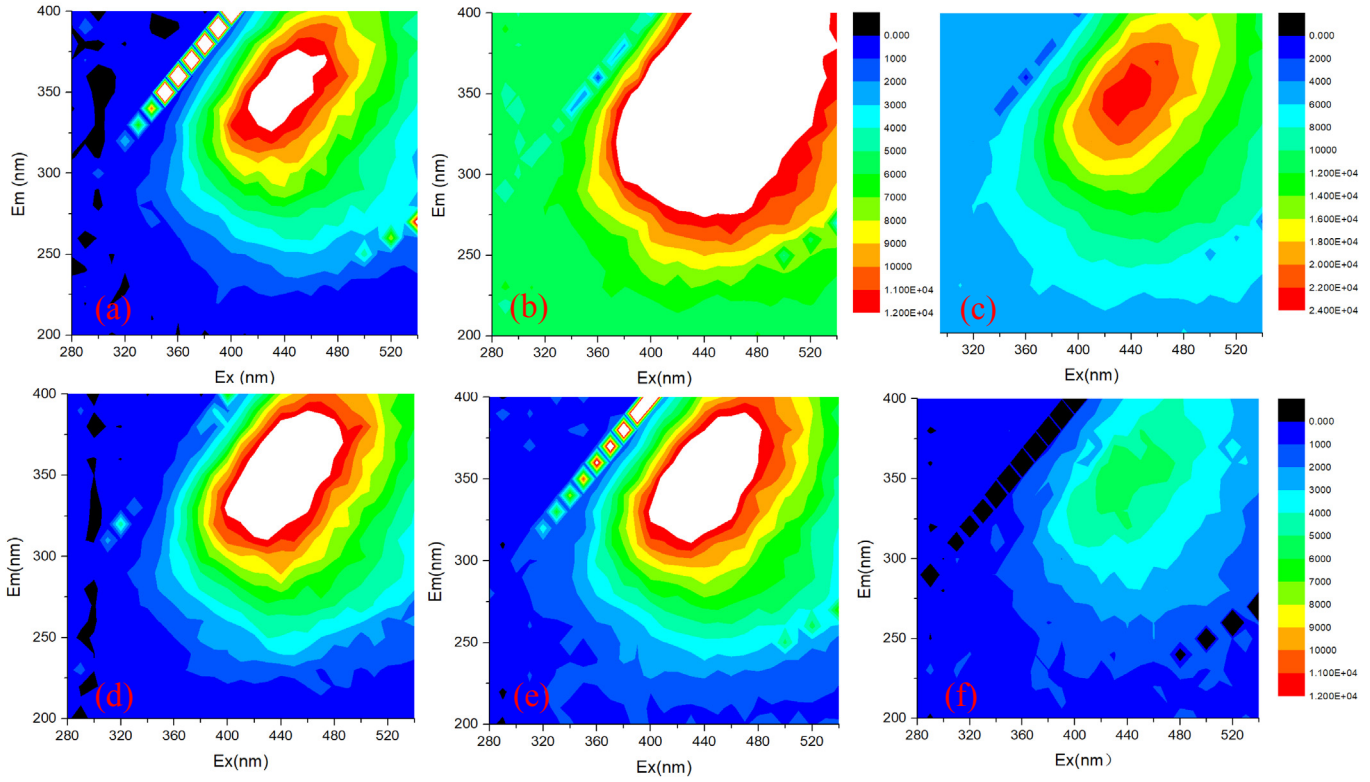

Fig. 3. The FEEM of digestate supernatant after 59 days' anaerobic digestion (diluted 10 times, (a) R0-Blank, (b)R1-Control at intensity 12,000, (c) R1-Control at intensity 24,000, (d) R2-MP1, (e) R5-MP4, (f) R9-MP8).

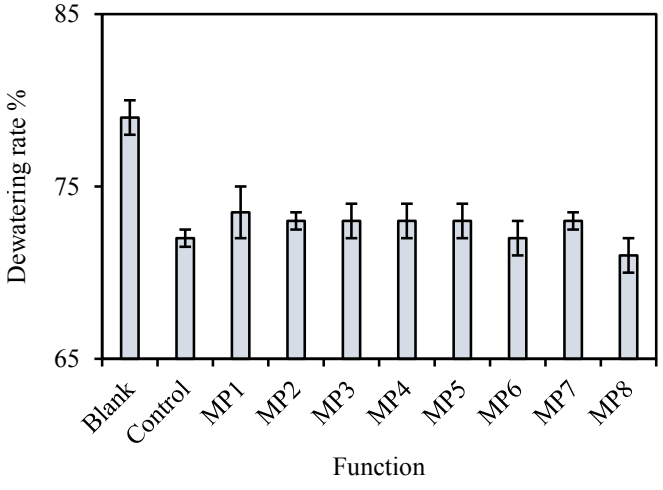

Fig. 4. The dewatering rate of digested sludge under various microplastics abundance.

3.4. The effects of microplastics abundance on microbial community

The microbial community compositions in the control reactor (R1) before and after digestion and the experimental reactors (R5 and R9) after digestion were analyzed and compared. The number of operational taxonomic units (OTUs) of raw sludge in R1 and digestate in R1 and R9 were 1924 ± 25, 1847 ± 15, and 1810 ± 28, respectively. The microplastics (R9 with microplastic PES abundance of 200,000 particles/kg·TS) insignificantly affected the microbial community composition ($p > 0.05$). The alpha diversity results also indicated that the existence of microplastics insignificantly affected the Chaos index (R1 *vs* R9, 2074 ± 31 *vs* 2113 ± 8) and Shannon index (R1 *vs* R9, 6.663 ± 0.057 *vs* 6.973 ± 0.043). The PD whole trees index values were 129 ± 1 and 128 ± 1 in R1 and R9, respectively. The observed species in R1 and R9 were 1537 ± 8 and 1565 ± 20, respectively. Therefore, the addition of microplastics insignificantly affected the structure and diversity of the microbial community.

Fig. 5 displays the microbial population phylum level distribution. The most abundant bacteria of raw sludge were *Proteobacteria, Bacteroidetes, Chloroflexi,* and *Gemmatimonadetes*. They accounted for over 75% of the total bacteria community in the raw sludge before digestion. The most abundant bacteria in R1, R5, and R9 were similar, including *Proteobacteria, Chloroflexi, Acidobacteria, Chloacimonetes, Bacteroidetes, Spirochaetes,* and *Acetothermia*. They accounted for over 80% of the total bacteria community in the digestate. The microbes in those phyla are widely reported as effective in converting organic compounds into volatile fatty acid (VFA) (Ariesyady et al., 2007; Zheng et al., 2013).

Compared with the control (R1), the experimental reactors with microplastics (R5 and R9) only showed a slight decrease in *Chloacimonetes* and a slight increase in *Chloroflexi*. In addition to the bacterial sequences, the Archaea sequences of the phylum *Euryarchaeota* were detected in raw sludge, R1, R5, and R9. The *Euryarchaeota* in R1, R5, and R9 was slightly higher than that in the raw sludge, but the difference was insignificant. Methanogens are reported to be a part of *Euryarchaeota*. The microbial community analysis indicated that the existence of microplastics insignificantly ($p > 0.05$) changed the community structure or the methanogens. (Wei et al., 2019a).

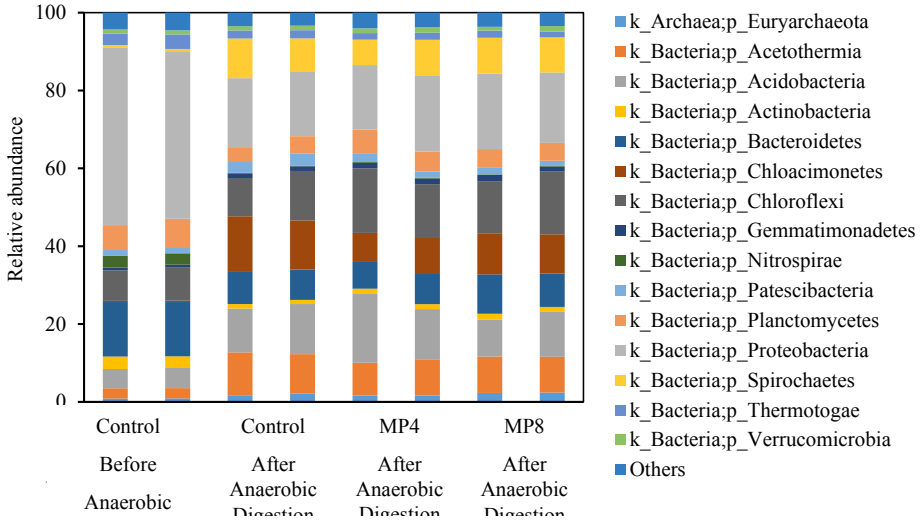

Fig. 5. The microbial community structure of different reactors.

3.5. Research perspectives

This study investigated the effects of the frequently detected microplastic PES in the methane production in conventional anaerobic digestion. Microplastics are abundant in activated sludge. Their effects on the most important resource recovery technology, that is, anaerobic digestion process, should be explored. The microplastic contamination in the WWTPs and sludge is inevitable. Some earlier works have pointed out that some specific microplastics can release toxic compounds and can thus inhibit the anaerobic digestion process. The effects of commonly found inertia microplastic PES were investigated to provide a better general view of the effects of common microplastics on the anaerobic digestion process. This study also could provide information on the control or mitigation of the effects posed by microplastics to alleviate their negative effects on the conventional anaerobic digestion process. Additional information for technology upgrade or policy adjustment in reducing the negative effects caused by microplastics on the wasted sludge resource recovery process could be provided as well.

Microplastics were abundant in terms of concentration, size, material, and other parameters. The different abundances, materials, and sizes could exert various effects on the anaerobic digestion process, and the combined effects should also be considered. The correlation between the bacteria and microplastics could provide useful information. The effects of microplastics on the activated sludge anaerobic digestion process performance, the mechanisms, and response to different treatment technologies should be further investigated. The negative effects caused by microplastics could be mitigated through the effective understanding of the relation between microplastics and anaerobic digestion process.

4. Conclusions

This study investigated the effect of common microplastic PES abundances on the anaerobic digestion methane production. The results indicated that the methane production with microplastics at abundances of 0–200,000 particles/kg·TS was inhibited as compared with that of the control. The one-substrate model indicated that, with microplastics, the hydrolysis rate (k) and BMP (B_0) decreased, but no linear correlation was found among k, B_0, and microplastic abundance in tests R1-R9. The digestate supernatant character results showed that the occurrence of microplastics could cause incomplete digestion and could thus slightly inhibit the methane production and cause higher amounts of retained organic matters and nutrients. The microbial community results indicated that the addition of microplastics insignificantly affected the microbial population structure. The microplastics added has shown slightly inhibition to the anaerobic digestion process and slight improvement in dewaterability.

Declaration of interest statement

The authors declare no conflict of interests.

CRediT authorship contribution statement

Lu Li: Formal analysis, Writing - original draft. **Shixiong Geng:** Methodology. **Zhouyang Li:** Data curation. **Kang Song:** Funding acquisition, Supervision.

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References

Altaş, L., 2009. Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. J. Hazard Mater. 162 (2–3), 1551–1556.
Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., 1976. Inhibition of nitrification by ammonia and nitrous-acid. J. Water Pollut. Control Fed. 48 (5), 835–852.
APHA, AWWA, WEF, Washinton, D.C., 2012. Standard Methods for the Examination of Water and Wastewater, twenty-second ed. American Public Health Association, USA, ISBN 978-087-553-013-0.
Ariesyady, H.D., Ito, T., Okabe, S., 2007. Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. Water Res. 41 (7), 1554–1568.
Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182.
Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99 (10), 4044–4064.

Chen, J.L., Ortiz, R., Steele, T.W., Stuckey, D.C., 2014. Toxicants inhibiting anaerobic digestion: a review. Biotechnol. Adv. 32 (8), 1523–1534.
Cheung, P.K., Fok, L., 2017. Characterisation of plastic microbeads in facial scrubs and their estimated emissions in Mainland China. Water Res. 122, 53–61.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597.
Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82.
Fu, S.F., Ding, J.N., Zhang, Y., Li, Y.F., Zhu, R., Yuan, X.Z., Zou, H., 2018. Exposure to polystyrene nanoplastic leads to inhibition of anaerobic digestion system. Sci. Total Environ. 625, 64–70.
Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr. Boil. 23 (19), 867–868.
Kang, H.J., Park, H.J., Kwon, O.K., Lee, W.S., Jeong, D.H., Ju, B.K., Kwon, J.H., 2018. Occurrence of microplastics in municipal sewage treatment plants: a review. Environ. Health Toxicol. 33 (3).
Karapanagioti, H.K., 2017. Microplastics and Synthetic Fibers in Treated Wastewater and Sludge. Wastewater and Biosolids Management. IWA Publishing, London, pp. 77–88.
Lares, M., Ncibi, M.C., Sillanpää, M., Sillanpää, M., 2018. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res. 133, 236–246.
Lee, H., Kim, Y., 2018. Treatment characteristics of microplastics at biological sewage treatment facilities in Korea. Mar. Pollut. Bull. 137, 1–8.
Leslie, H.A., Brandsma, S.H., Van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133–142.
Li, X., Dai, X., Takahashi, J., Li, N., Jin, J., Dai, L., Dong, B., 2014. New insight into chemical changes of dissolved organic matter during anaerobic digestion of dewatered sewage sludge using EEM-PARAFAC and two-dimensional FTIR correlation spectroscopy. Bioresour. Technol. 159, 412–420.
Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y., 2018. Microplastics in sewage sludge from the wastewater treatment plants in China. Water Res. 142, 75–85.
Li, L., Liu, D., Song, K., Zhou, Y., 2019. Performance evaluation of MBR in treating microplastics polyvinylchloride contaminated polluted surface water. Mar. Pollut. Bull. 110724.
Liu, X., Xu, Q., Wang, D., Zhao, J., Wu, Y., Liu, Y., et al., 2018. Improved methane production from waste activated sludge by combining free ammonia with heat pretreatment: performance, mechanisms and applications. Bioresour. Technol. 268, 230–236.
Luo, K., Yang, Q., Li, X.M., Chen, H.B., Liu, X., Yang, G.J., Zeng, G.M., 2013. Novel insights into enzymatic-enhanced anaerobic digestion of waste activated sludge by three-dimensional excitation and emission matrix fluorescence spectroscopy. Chemosphere 91 (5), 579–585.
Ma, H., Guo, Y., Qin, Y., Li, Y.Y., 2018. Nutrient recovery technologies integrated with energy recovery by waste biomass anaerobic digestion. Bioresour. Technol. 269, 520–531.
Mahon, A.M., O'connell, B., Healy, M.G., O'connor, I., Officer, R., Nash, R., Morrison, L., 2016. Microplastics in sewage sludge: effects of treatment. Environ. Sci. Technol. 51 (2), 810–818.
Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. Renew. Sustain. Energy Rev. 45, 540–555.
Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., et al., 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environ. Pollut. 218, 1045–1054.
Mintenig, S.M., Int-Veen, I., Löder, M.G., Primpke, S., Gerdts, G., 2017. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res. 108, 365–372.
Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50 (11), 5800–5808.
Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Mar. Pollut. Bull. 112 (1–2), 39–45.
Pijuan, M., Wang, Q., Ye, L., Yuan, Z., 2012. Improving secondary sludge biodegradability using free nitrous acid treatment. Bioresour. Technol. 116, 92–98.
Raju, S., Carbery, M., Kuttykattil, A., Senathirajah, K., Subashchandrabose, S.R., Evans, G., Thavamani, P., 2018. Transport and fate of microplastics in wastewater treatment plants: implications to environmental health. Rev. Environ. Sci. Biotechnol. 1–17.
Song, K., Zhou, X., Liu, Y., Xie, G.J., Wang, D., Zhang, T., et al., 2016. Improving dewaterability of anaerobically digested sludge by combination of persulfate and zero valent iron. Chem. Eng. J. 295, 436–442.
Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C., Ni, B.J., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37.
Talvitie, J., Mikola, A., Koistinen, A., Setälä, O., 2017. Solutions to microplastic pollution–Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Res. 123, 401–407.
Vasco-Correa, J., Khanal, S., Manandhar, A., Shah, A., 2018. Anaerobic digestion for bioenergy production: global status, environmental and techno-economic implications, and government policies. Bioresour. Technol. 247, 1015–1026.
Wang, Q., Ye, L., Jiang, G., Jensen, P.D., Batstone, D.J., Yuan, Z., 2013. Free nitrous acid (FNA)-based pretreatment enhances methane production from waste activated sludge. Environ. Sci. Technol. 47 (20), 11897–11904.
Wang, Q., Wei, W., Gong, Y., Yu, Q., Li, Q., Sun, J., Yuan, Z., 2017. Technologies for reducing sludge production in wastewater treatment plants: state of the art. Sci. Total Environ. 587, 510–521.
Wang, D., Liu, B., Liu, X., Xu, Q., Yang, Q., Liu, Y., et al., 2018. How does free ammonia-based sludge pretreatment improve methane production from anaerobic digestion of waste activated sludge. Chemosphere 206, 491–501.
Wei, W., Huang, Q.S., Sun, J., Wang, J.Y., Wu, S.L., Ni, B.J., 2019a. Polyvinylchloride microplastics affect methane production from the anaerobic digestion of waste activated sludge through leaching toxic bisphenol-A. Environ. Sci. Technol. 53 (5), 2509–2517.
Wei, W., Zhang, Y.T., Huang, Q.S., Ni, B.J., 2019b. Polyethylene terephthalate microplastics affect hydrogen production from alkaline anaerobic fermentation of waste activated sludge through altering viability and activity of anaerobic microorganisms. Water Res. 163, 114881.
Xu, Q., Li, X., Ding, R., Wang, D., Liu, Y., Wang, Q., et al., 2017. Understanding and mitigating the toxicity of cadmium to the anaerobic fermentation of waste activated sludge. Water Res. 124, 269–279.
Xu, Q., Liu, X., Wang, D., Wu, Y., Wang, Q., Liu, Y., et al., 2018. Free ammonia-based pretreatment enhances phosphorus release and recovery from waste activated sludge. Chemosphere 213, 276–284.
Zhen, G., Lu, X., Kato, H., Zhao, Y., Li, Y.Y., 2017. Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: current advances, full-scale application and future perspectives. Renew. Sustain. Energy Rev. 69, 559–577.
Zheng, X., Su, Y.L., Li, X., Xiao, N.D., Wang, D.B., Chen, Y.G., 2013. Pyrosequencing reveals the key microorganisms involved in sludge alkaline fermentation for efficient short-chain fatty acids production. Environ. Sci. Technol. 47 (9), 4262–4268.
Ziajahromi, S., Neale, P.A., Leusch, F.D., 2016. Wastewater treatment plant effluent as a source of microplastics: review of the fate, chemical interactions and potential risks to aquatic organisms. Water Sci. Technol. 74 (10), 2253–2269.