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Comprehensive *meta*-analysis reveals the impact of non-biodegradable plastic pollution on methane production in anaerobic digestion

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ABSTRACT

Plastics as emerging contaminants have been heavily accumulated in organic wastes (e.g. waste activated sludge and food waste), which have a dramatically different impact on the resource recovery from these organic wastes through anaerobic digestion. However, the reported studies differ significantly from each other, and a comprehensive analysis to reveal the complex effects of plastic pollution in organic wastes on methane production is still lacking. In this study, 28 articles were selected from three citation databases for *meta*-analysis according to the preferred reporting items for systematic reviews and *meta*-analyses guidelines. Subgroup analysis was then performed to determine the effects of plastic type, particle size, and concentration on methane production and multiple physicochemical parameters. The *meta*-analysis showed that the mean effect size of plastic pollution on methane production was 0.93 [0.89, 0.98] ($p < 0.05$). The results also revealed that the presence of plastics negatively affected the organic content and enzyme activity, as well as increasing the reactive oxygen species. In addition, the effect of nanoplastics on these physicochemical parameters was more significant compared to microplastics, like highlighted by most studies. Finally, structural equation modelling quantified that plastic pollution affected methane production by two main pathways: inhibition of organic solubilisation and induction of reactive oxygen species. This information is helpful to a more profound understanding the underlying toxicity mechanisms of plastic pollution to methane production and provide guidance for future research.

1. Introduction

The word 'plastics' brings together a wide range of synthetic or semi-synthetic polymer materials derived mainly from fossil fuel. The majority of fossil-based plastics can persist in the environment for long periods due to their stable backbone polymer chains, including high resistance to microbial degradation [1,2]. Therefore, these fossil-based plastics are also referred to as non-biodegradable plastics. In this paper, 'plastics' was used for simplicity to mean the fossil-based plastics. Since being invented in the late 19th century, the use of plastics in our everyday lives has increased rapidly and exponentially. It was estimated that annual production of plastics has reached 359 million tons in 2018, with approximately 8.3 billion tons produced over the last 70 years [3,4]. The prevalence of plastics in the natural environment has been an emerging problem because of the discarding of plastics during the production and usage of various anthropogenic activities, and the failure of today's waste management systems to handle plastic waste effectively [5–7]. Most of these plastics in domestic and industrial wastewater will eventually enter the wastewater treatment system, and more than 90 % of plastics have been found to be retained in waste activated sludge (WAS) [8]. In fact, WAS usually contains lots of organic matter, such as carbohydrates and proteins, making it a valuable substrate for recycling resources and sustainable energy [9]. Moreover, plastics widely used in food packaging will inevitably enter food waste due to the imperfect garbage classification [10]. These solid wastes have a high content of biodegradable organic matter and moisture, which could generate serious odour and leachate if not treated in a prompt way, resulting in potential ecological pollution.

Abbreviations: AD, anaerobic digestion; WAS, waste activated sludge; MPs, microplastics; NPs, nanoplastics; PVC, polyvinyl chloride; PE, polyethylene; PA6, Polyamide 6; PC, polycarbonate; PS, polystyrene; PEI, polyethyleneimine; PES, polyester; PET, polyethylene terephthalate; PP, polypropylene; SEM, structural equation model; VFAs, volatile fatty acids; sCOD, soluble chemical oxygen demand; AK, acetate kinase; BK, butyrate kinase; F420, coenzyme F420; ROS, reactive oxygen species.

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Anaerobic digestion (AD) has been considered as the dominant treatment process currently, as it combines the removal of organic wastes with the generation of renewable resources [11]. However, AD involves complex biochemical processes in which a wide range of anaerobic microbes work together, and it is inherently less stable and susceptible to adverse factors [12]. As an emerging contaminant, plastics can cause biotoxicity through the release of toxic monomers and plastic additives [13]. Specifically, plastic products contain a variety of chemicals, such as plasticizers, stabilizers, antioxidants, and fire retardants, which can enhance their desired properties [14]. However, these plastic additives are not chemically bound to the plastic matrix, resulting in their release into the environment through abrasion, leaching, and dissolution processes [14,15]. Moreover, plastics are gradually fragmented into microplastics (MPs) through various natural processes, like mechanical impact, heat, light, or biodegradation. These MPs can be ingested by organisms and accumulated in their bodies, leading to physical harm, disrupting physiological processes, and triggering inflammatory responses [16]. Furthermore, MPs and nanoplastics (NPs) with high specific surface area may constitute a carrier for a variety of pollutants, such as heavy metals or organic pollutants [17,18]. It was reported that cadmium at 5 mg/g total suspended solids decreased methane production by 30.3 % in the AD of WAS compared to the blank group [19]. Therefore, plastic polluted organic wastes may interfere with methane production in the AD process, bringing new challenges to the AD system operation.

Recently, several studies have emphasized that certain types of plastics have a potential adverse effect on AD. For example, polyvinyl chloride (PVC) MPs negatively affected the AD of WAS by leaching toxic bisphenol A [20]; the negative effect of polyethylene (PE) MPs was probably attributed to the induction of reactive oxygen species (ROS) [21]. Based on microbial community and enzyme activity analysis, Wang et al. [22] revealed that the main inhibition mechanism of various MPs was oxidative stress induced by the leachate, which destroyed microbial cells and reduced microbial activity. However, the effects of plastics on AD have not always been negative across studies. Polyamide 6 (PA6) enhanced methane production due to the leaching of caprolactam, which promoted the activity of key enzymes [23]. It has also been shown that the effect of plastics on AD could depend on their particle size and concentration. Polycarbonate (PC) MPs at 30 particles/g of total solids improved methane production by 24.7 %, but higher doses of PC had a significant inhibitory effect on AD [24]. In general, despite numerous reports on the effects of plastics on AD, comprehensive insights into the role of plastics in the AD process are still scarce, and the overall potential impact of plastic pollution in AD remains elusive to be predicted accurately.

In the present study, we investigated and collected literature data concerning the effects of plastics on AD. According to these data, a *meta*-analysis was conducted to compare methane production in AD with and without plastic pollution, and the significance of plastic types, particle sizes and concentration ranges were evaluated separately for more details. We also assessed the potential toxicity of plastics toward various parameters during AD, including variations in the content of multiple organic substances and changes in enzyme activity. Meanwhile, a structural equation model (SEM) was employed to quantitatively evaluate the key influencing factors and related mechanisms of plastics interruption to AD at the physiological level. To our knowledge, this study is the first to systematically quantify the effect of plastics on AD operation using a comprehensive *meta*-analysis. Furthermore, the obtained results offer valuable insights and contribute to new suggestions and priorities for future relevant studies.

2. Materials and methods

2.1. Literature search

The literature search was conducted by two separate individuals according to the preferred reporting items for systematic reviews and *meta*-analyses guidelines, in the databases Web of Science, Scopus, and PubMed (prior to 15 July 2023). The following search terms were employed: (“Plastic” OR “Macroplastic” OR “Microplastic” OR “Nanoplastic” OR “Polyethylene” OR “Polypropylene” OR “Polyvinyl chloride” OR “Polystyrene” OR “Polyethylene terephthalate”) AND “Anaerobic digestion’’ AND “Methane”, and the searches in Web of Science and Scopus were limited to the topic [title/abstract/keywords] and in PubMed to [title/abstract]. A rigorous search strategy was developed and implemented to collect the most relevant, novel, and credible datasets (Fig. 1). The initial search yielded 538 research articles, and the list was narrowed to 28 articles by stipulating the following criteria (Table S1): (1) the studies investigated the impact of plastics on AD; (2) a control or plastic-free treatment was conducted; (3) the results included methane production data; and (4) measurable data were presented for the determination of mean value and uncertainty of methane yield, as standard deviation or standard error. The current *meta*-analysis excluded review articles or those articles that quantified the impact of biodegradable plastics on methane production, which exceeded the primary objectives of this study.

2.2. Main data extraction

The raw data were extracted directly from the texts or tables of the publications, and the GWebPlotDigitizer (https://automeris.io/WebPlotDigitizer/, Version 4.6) was used to extract the data from the figures. For each selected study, the following original information was collected as input data: plastic particle sizes; plastic concentrations; plastic types, which included polystyrene (PS), PA6, PC, PE, polyethyleneimine (PEI), polyester (PES), polyethylene terephthalate (PET), polypropylene (PP), and PVC; feedstock types; feedstock to inoculum ratio based on volatile solid; pH; temperature; bioreactor volume; working volume; the ratio of working volume to bioreactor volume. The methane production was collected as endpoint parameter. In addition, to explore the mechanism of plastic influence on AD, a variety of physicochemical parameters during the AD processes were gathered. If articles were unavailable for partial data, the study was excluded from the corresponding data analysis.

2.3. Meta-analysis

Three essential results were extracted from the screened papers: the mean, standard deviation, and the number of replicates. The value of error bars was assumed to be standard deviations if not stated in the paper, and the standard deviation was estimated to be 10 % of the mean when it was missing [25]. If only the standard error was provided, standard deviation was calculated according to Zhang et al.[26]. Before further analysis, a subgroup analysis was also conducted for analysing the effect of plastic on methane yield. To explain the variation in response of methane yield, plastic particle size was divided into three groups: >5 mm (macroplastics), >1 μm and ≤ 5 mm (MPs), and ≤ 1 μm (NPs). As the units of concentration used in the article are not uniform (like mg/L, particles/g of total solids, and mg/g of volatile solids, etc.), the most frequently used unit (mg/L) was selected for further analysis. Additionally, the plastic concentrations were classified as high (>100 mg/L), moderate (>1 mg/L and $\leq$ 100 mg/L), and low (≤ 1 mg/L). Subsequently, single *meta*-regression models were created using the continuous variables, i.e., plastic particle size (mm) and concentration (mg/L), to quantify the different effects of plastics on methane production from AD. Finally, publication bias was assessed using a contour-enhanced funnel plot approach (Fig. S1). In general, *meta*-analysis was conducted using the “metafor” package and “forestplot” package, implemented in R version 4.1.3 (https://www.r-project.org/), and the methods of *meta*-analysis referred to previous studies [26,27].

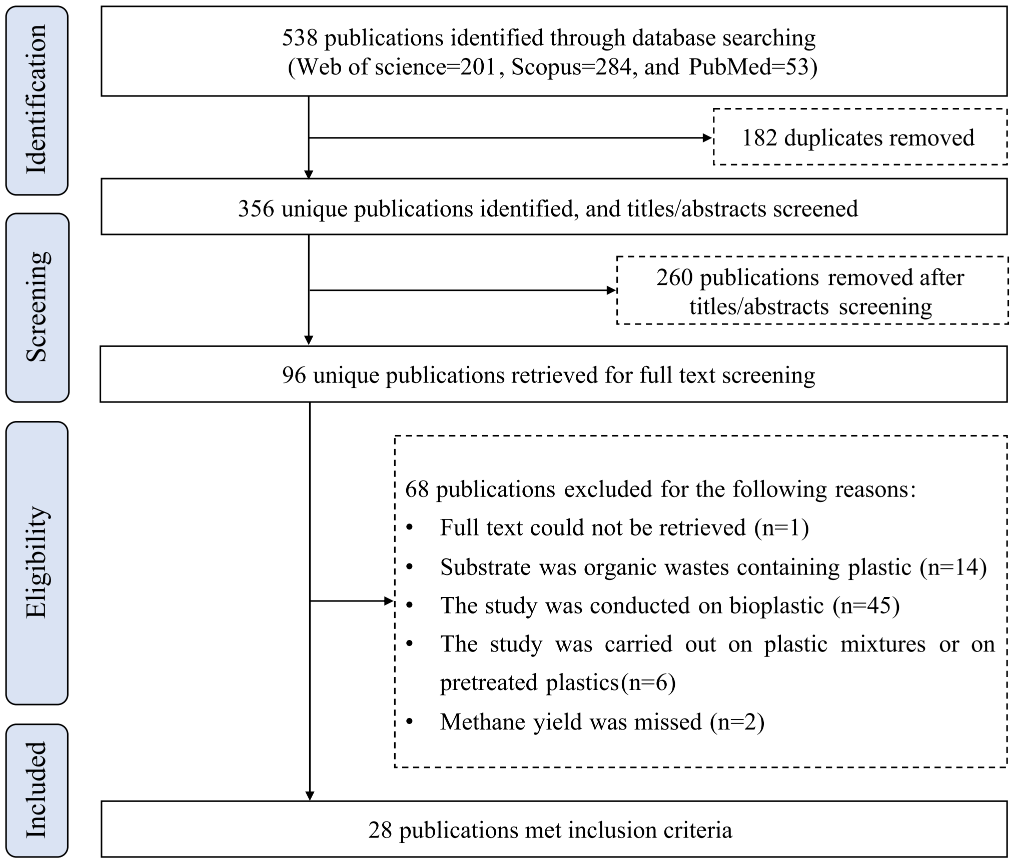

Fig. 1. Preferred reporting items for systematic reviews and *meta*-analysis (PRISMA) flowchart of the publication identification and selection process.

2.4. Structural equation model

The SEM was established to assess the hypothetical response of plastic pollution to methane production potential and different parameters during AD. SEM is a statistical model that is primarily used to evaluate whether theoretical models are plausible when compared to observed data. Firstly, the Mantel test with vegan package was used to analyse the data for all variables, including plastic particle size, plastic concentration, bacterial diversity (Shannon's indices), ROS, soluble chemical oxygen demand (sCOD), protein, polysaccharide, volatile fatty acids (VFAs), protease, α-glucosidase, acetate kinase (AK), butyrate kinase (BK), coenzyme F420 (F420) and methane production. This analysis was conducted to establish the pairwise correlations among these variables. A hypothetical model was drawn based on the correlation between these variables and the methane production pathway during the AD process [18,23]. To construct the SEM, datasets containing null values were deleted, ensuring that all variables were included in the analysis with complete data. The SEM software package AMOS (Version 24, IBM Corporation, USA) was employed to develop the model using the maximum-likelihood estimation method. To assess the fitness of the SEM, non-significant chi-square test ($P > 0.05$), high goodness-of-fit index (GFI > 0.90), and low root-mean-square errors of approximation (RMSEA < 0.05) were employed as criteria. Standardized pathway coefficients were applied to indicate the relative effect of one variable on another. Finally, the indirect, direct, and overall effects of each variable on the methane production potential were calculated.

3. Results and discussions

3.1. Overview of the collected papers

Initially, a total of 28 research articles were identified from the 538 publications available up to July 2023, all of which conducted experimental investigations on the effects of plastic pollution on AD. Since 3 publications in 2018, research on plastic toxicity in AD has attracted increasing attention, with a total of 28 articles published by 2023 (Fig. 2a). This trend would likely continue considering the occurrence of plastic pollution worldwide, the increasing importance of renewable energy, and growing scientific interest and funding. Therefore, it is expected that an increasing number of publications will be issued in the coming years.

In terms of the types of plastics employed in the studies, more than one-third of the samples (38.6 %) were PS, 16.6 % focused on PVC, 15.2 % on PE, and only 3.4 % on PEI (Fig. 2b). PS particle was chosen as a representative plastic in most studies since it is one of the most common plastic pollutants in the environment [28]. Meanwhile, the loose structure and rough surface of PS materials make them more likely to flake off under slight mechanical forces, resulting in many crushed points with a size range of 20–100 μm on the materials surface. Recently, the influence of various types of plastics on AD has been reported. For example, the cumulative methane production was reduced by 12 % when exposed to PES at 1000 particles/kg of activated sludge, and this reduction can reach 23 % with PS pollution at 100 mg/L [29,30]. In contrast, PA6 increased methane yield by enhancing key enzyme activities through caprolactam leaching [23]. These divergent results suggest that different types of plastic may exhibit varying effects on AD processes due to their distinct physicochemical properties, which cannot be explained by similar mechanisms. Hence, the plastic types are critical parameters that need to be addressed when assessing the potential toxicity of plastics to AD.

Plastics in the environment are classified into three categories based on their size, those > 5 mm are called macroplastics; those < 5 mm are defined as MPs; and smaller sizes ranging from 1 nm to 1 μm are classified as NPs [31]. Only 5.5 % of the datapoints focused on the combined

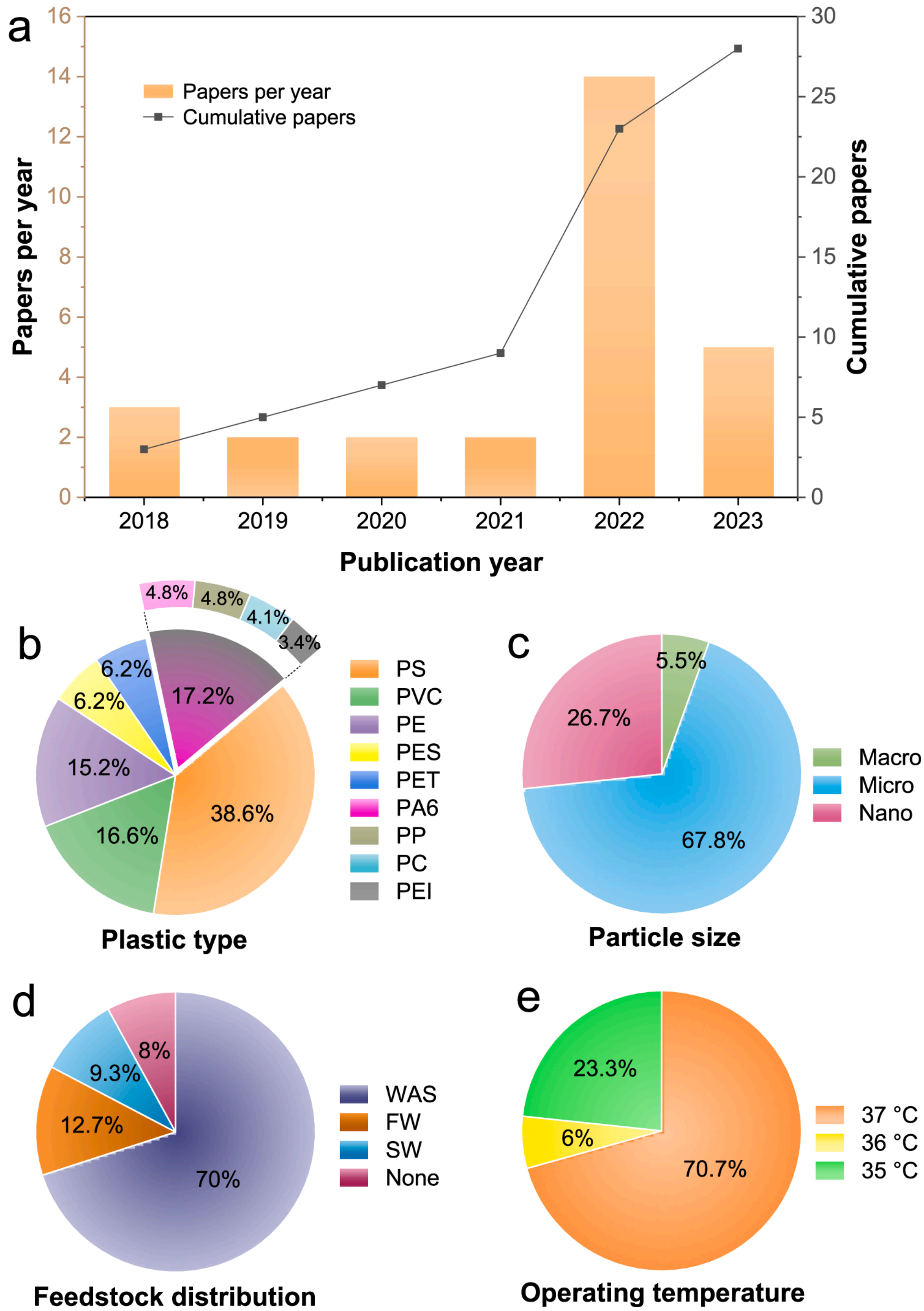

Fig. 2. Basic information statistics of the selected literatures. (a) Number of publications that experimentally examined the effects of plastic on anaerobic digestion. (b–e) Respective proportion of plastic type, particle size, feedstock distribution, and operating temperature. PVC: polyvinyl chloride; PE: polyethylene; PA6: Polyamide 6; PC: polycarbonate; PS: polystyrene; PEI: polyethyleneimine; PES: polyester; PET: polyethylene terephthalate; PP: polypropylene; Macro: macroplastics; Micro: microplastics; Nano: nanoplastics; WAS: waste activated sludge; FW: food waste; SW: synthetic wastewater; None: none (only inoculum).

impacts of macroplastics on methane yield from AD (Fig. 2c). Plastic particle size is decisive in the generation of toxicity, and it is natural to assume that MPs and their degradation products in the form of NPs have the potential to cause serious damage to cells [32]. However, Wang et al. investigated the toxicity of PS MPs on anaerobic granular sludge, and revealed that larger particles exhibited stronger dispersing properties, increasing their exposure to the sludge and ultimately reducing biological activity [33]. What's more, plastic particle sizes have a significant impact on the potential contact area with functional microbials, which subsequently leads to different effects on AD process [10,21]. In general, plastics with smaller particle sizes usually have a larger surface area per unit mass, which allows a larger proportion of reactive groups displayed on their surfaces, thus generating ROS through free radical and catalytic reactions with molecular dioxygen.

Due to the ubiquitous and growing production, usage, degradation, and disposal of plastic items, MPs are consistently being released into the environment. Wastewater treatment plants are receptors for MP pollution, and significant amounts of MPs can be detected in wastewater and WAS [34,35]. As the terminal receiver in wastewater treatment plants, WAS was the most studied substrate to explore the effect of plastics on AD (Fig. 2d). The global MPs concentrations in the influent and sludge ranged from 1 to 10,044 particles/L and 400 to 7,000 particles/kg of wet weight, respectively [36]. From our collected data, the concentrations of MPs and NPs in experiments were 0.05–1000 mg/L and 0.05–250 mg/L respectively, based on 34 observations. In addition, the waste plastic materials widely used in food packaging inevitably enter the food waste due to poor waste classification, and these contaminated food waste may release smaller plastic particles during the food waste recycling process [10,37]. Food waste was employed as a substrate in 12.7 % of studies (Fig. 2d). All data collected employed the mesophilic conditions (30–40 °C), including 35 °C (70.7 %), 36 °C (6 %), and 37 °C (23.3 %) (Fig. 2e).

3.2. General trends of plastic effect on methane production

As the parameters of the AD system also have great impacts on methane production, *meta*-analyses were first performed for them (Fig. S2). The changes in feedstock to inoculum ratio, pH, and bioreactor volume led to fluctuations in the response ratio results, suggesting that these indicators should be focused on in future AD. Summarizing across the selected categories, the presence of plastic greatly inhibited methane yield by 7 % with response size of 0.93 [0.89, 0.98] ($p < 0.05$). The PS showed the highest response size of 0.90 [0.86, 0.93] implying its greater effect on AD. PVC, PE, PES, and PEI somehow showed almost similar levels of response ratio (0.91, 0.91, 0.92, and 0.92, respectively) (Fig. 3). Several studies have shown that the presence of PS, PVC, and PE MPs in WAS may release toxic additives or cause redox damage, thus limiting methane production in AD [20,38,39]. These different types of plastics work through various interaction mechanisms to inhibit AD. For example, PS NPs interfered with the digestion process by inducing ROS and thus inhibiting critical enzyme activities [40]. While PVC, as the most toxic plastic throughout its production and disposal, was mainly responsible for the leaching of toxic substances such as Bisphenol A to microorganisms and finally affected AD [20,41]. Plastics commonly contained hazardous additives such as flame retardants and phthalates, and could potentially absorb hydrophobic pollutants from the surrounding environment [42]. In addition, MPs are readily accessible to microorganisms in the environment because of their small particle size, large specific surface area, and high hydrophobicity [43]. In contrast, there was no significant difference of PA6 (n = 5, $p > 0.05$), PC (n = 5, $p > 0.05$), PP (n = 6, $p > 0.05$), and PET (n = 6, $p > 0.05$) on methane yield compared to that of control groups (Fig. 3). Furthermore, PA6, PC, and PP had a promoting effect on methane production from the overall response ratio results, which were 1.07, 1.07, and 1.16 respectively. The results might be explained by the insufficient amount of data from studies for these plastic types and the variation in the effect on AD at different plastic particle sizes and concentrations. Chen et al. observed that a dose-dependent influence of PC MPs on AD by monitoring the changes in methane yield at different PC concentrations [24]. Their results showed that PC MPs with 10–60 particles/g of total solids increased the methane yield, while 200 particles/g of total solids decreased the methane yield by 8.09 ± 0.1 %. However, some studies found that plastic pollution can boost methane production, which may lead to fluctuations in meta results. For instance, PA6 MPs can motivate methane yield from AD of WAS by promoting acidification and methanogenesis [23]. Furthermore, a mixture of plastics can be found in the sludge of practical AD systems, whereas the screened literatures on the subject was not sufficient to complete *meta*-analyses. Based on the composition of MPs in the sludge, 75 mg/L of PET (36 %), PS (15 %), PE (42 %) and PP (8 %) was added to the anaerobic granular sludge and the study revealed that coexisting MPs reduced methane production by 15.9 % compared to the control group [44].

In the study of the plastics toxicity to environmental organisms, the particle size was a frequently examined indicator. These studies have been extensively reviewed and the results indicated that smaller plastic particle size was more toxic to the organisms and had more adverse effects on the environment than larger plastic particle size [7,45]. The same results were found in our study, where NPs were significantly reduced methane production by 12 % with response ratio of 0.88 [0.84, 0.93] ($p < 0.05$), while MPs and macroplastics resulted in reductions of 4 % and 7 %, respectively (Fig. 4). By investigating the size-dependent effects of PS MPs on AD performance of food waste, Li et al. found that the smaller the plastic particle size, the higher the inhibitory effect on methane yield [10]. The further physicochemical analyses showed that small size of MPs induced more ROS leading to cellular toxicity and inhibited the activities of key enzymes (protease, α-glucoside, BK, AK, and F420), ultimately decreasing methane yield. Moreover, this study evaluated the effect of plastics on methane production based on plastic

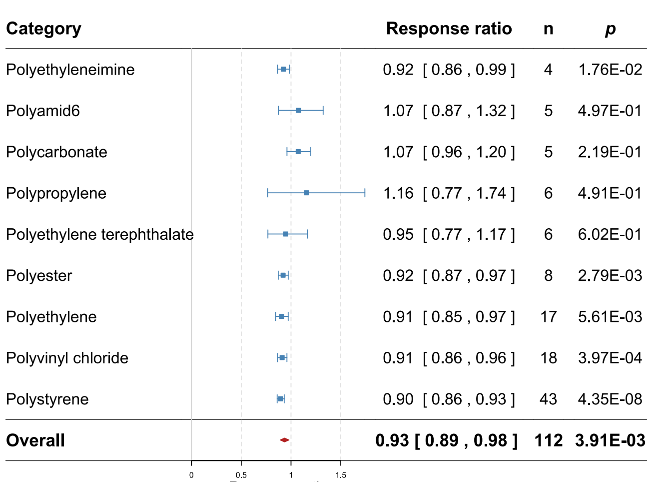

Fig. 3. The effects of plastic types on methane production. The blue square symbols show mean effect size with error bars representing 95 % confidence interval, and the red diamond represents the summary effect. A ratio < 1 indicates that the response from the treatment (including plastic) is lower compared to the control group. n refers to sample size, and *p* means the *p*-value of the Q test with $p < 0.05$ indicating a significant difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

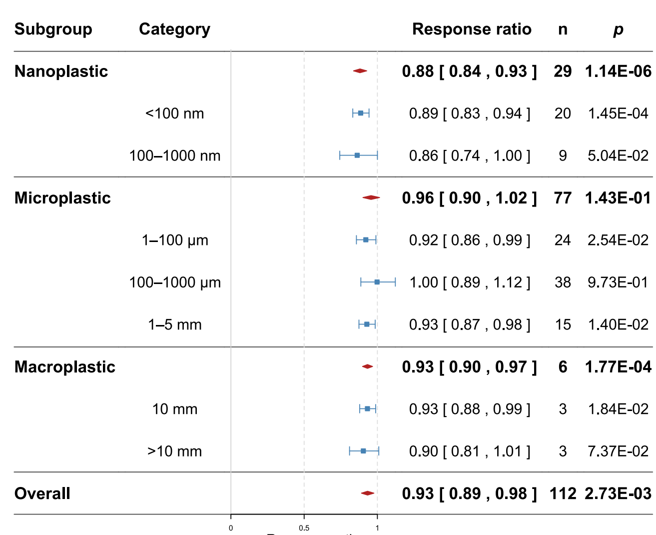

Fig. 4. The effects of plastic particle sizes on methane production. The blue square symbols show mean effect size with error bars representing 95 % confidence interval, and the red diamond represents the summary effect. A ratio < 1 indicates that the response from the treatment (including plastic) is lower compared to the control group. n refers to sample size, and *p* means the *p*-value of the Q test with $p < 0.05$ indicating a significant difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

type and particle size, and the results showed that PS and PE had a greater effect on AD as the particle size decreased, resulting in less methane production (Fig. S3). Indeed, certain studies have shown that the effect of plastic particles to microbial community depended on the size. For example, Wang et al. found that 50 nm PS led to the enrichment of *Mariniphaga*, *Candidatus Microthrix*, *Brevefilum*, and *Perlabentimonas* by microbiome analysis, whereas 1 μm and 10 μm did not substantially shape the core bacterial of digested sludge [18]. Li et al. demonstrated that the relative abundance of methanogenesis-related microorganisms (especially *Methanosarcina*) was reduced by the addition of PS MPs, and this effect was enhanced as particle size decreased [10]. It is noteworthy that MPs with a particle size of 100–1000 μm did not exhibit a statistically significant difference in methane yield with response ratio of 1.00 [0.89, 1.12] ($p = 0.973$) (Fig. 4). Similarly, according to the toxicity of different plastic particle sizes to microalgae, some authors have suggested introducing a threshold size for the ecological risk assessment of plastics and considering particles above 100 μm as low threat [46].

Besides the plastic type and particle size, the plastic concentration is also an important parameter in AD. Because of differences in the units used for concentrations, we chose the most used units (mg/L) and finally obtained a total of 34 groups of data. The results on plastic concentration showed that low concentration (≤1 mg/L) and moderate concentration (>1 mg/L and ≤ 100 mg/L) significantly reduced methane yield by 14 % and 7 %, while high concentrations (>100 mg/L) showed no significant difference (Fig. 5). These results might be explained by the fact that high concentration levels in the studies corresponded to large particle sizes, e.g. a concentration of 1000 mg/L was associated with a particle size of 5 mm [47]. For further determination of the influence of plastic concentration on AD in the same particle size range, the most researched plastic, PS, was selected for analysis. As shown in Fig. S4, the inhibition of PS on methane yield did not vary much with concentration in the same particle size range, indicating that the plastic particle size may be more important for methane yield. This result was consistent to previous research where the difference in methane production can reach up to 37 % when only particle size was varied, while this difference was reduced to 21 % when only the concentration changed [10]. However, more research is still needed to fully substantiate this viewpoint.

3.3. Effects of plastic on various factors during anaerobic digestion

The production of methane from AD sequentially undergoes solubilisation, hydrolysis, acidification, acidogenesis, acetogenesis and methanogenesis [48,49]. The complex process of AD involves a wide range of organic matter transformations, microorganisms functioning at each stage, and enzymes responding to each stage. The overall effects of plastic on the various parameters are shown in Fig. 6 and Fig. S5, which include four organic compounds, five types of enzymes, and ROS. Specifically, the solubilisation efficiency is generally expressed in studies using the release of sCOD. These sCOD consists mainly of soluble proteins and soluble polysaccharides, which are hydrolysed into small molecular substances and subsequently generate VFAs by acidification. Then, VFAs are converted into acetic acid and eventually to methane through the processes of the acetogenesis and methanogenesis [24,48,49]. Our results showed that sCOD, protein, and polysaccharides all exhibited varying degrees of decrease in the presence of plastic (Fig. 6). In addition, plastics were able to significantly reduce VFAs production with response ratio of 0.82 [0.68, 0.99], which was consistent with the previous study [18]. Sludge solubilisation was primarily related to the particle size and the organic matter composition of sludge flocs [50]. It was reported that the median diameter of sludge floc particles increased with increasing PEI concentration, and a large sludge particle size implies a small surface area that will result in a low solubilisation [51]. The organic matter in the sludge was mainly isolated in extracellular polymeric substances, consisting mainly of proteins, polysaccharides, and humic substances [52]. The results of three-dimension excitation emission matrix fluorescence spectroscopy revealed that plastics can inhibit the release of proteins and carbohydrates from extracellular polymeric substances, while the transformation of organics in extracellular polymeric substances into the dissolved state was attenuated [51,53]. In general, plastics affected the solubilisation phase of AD through two main pathways, i.e. by facilitating the aggregation of sludge floc and by inhibiting the release of organics from extracellular polymeric substances.

Moreover, it is well known that the AD process requires the participation of a variety of enzymes, thus methane production is related directly to the key enzyme activities. As shown in studies, proteases and α-glucosidases firstly hydrolyse proteins and polysaccharides to amino acids and monosaccharides respectively. The produced amino acids are converted to VFAs via BK, and AK converts acetyl-CoA to acetic acid. Finally, acetic acid is methanated in the presence of F420 [18,23]. The effect of plastic existence on the activity of these key enzymes is shown in Fig. 6 and Fig. S5. In general, plastic reduced all enzyme activities except for BK, but none of the results showed statistically significant differences ($p > 0.05$). Plastic induced ROS is another pathway mechanism that affects methanogenesis. ROS could be generated in AD system at sub-micromolar oxygen concentration when exposed to adverse conditions [54]. As illustrated in Fig. 6, the addition of plastic substantially increased ROS generation, which confirmed the results of the previous studies [40]. ROS is an important indicator of cell viability, with excessive ROS exposure might causing toxic oxidative stress to cells, even leading to cell lysis and death [55]. Although potential inhibitory mechanisms for plastic proposed in the literatures include direct destruction of microbial cells, induction of ROS generation, inhibition of key enzymes and metabolic functions, and leaching of toxic chemicals and additives, the most significant of these inhibitory mechanisms remain unknown.

To further explore the effect of plastic on these parameters, *meta*-analyses of plastic particle size and type were carried out. Due to the lack of uniformity in the concentration units and the limited availability of data on these parameters (organic contents and enzyme activities) across the articles, there were not enough data to support concentration-based analyses. As shown in Fig. S6, the plastic types fluctuated greatly on the results. However, the wide range of plastic types could easily make the outcome based on a single literature analysis. Fig. 6 showed the effect of plastic particle size on various parameters during AD. Unsurprisingly, the MP category displayed no major differences from the overall results, but the NP category was quite unexpected. The results for

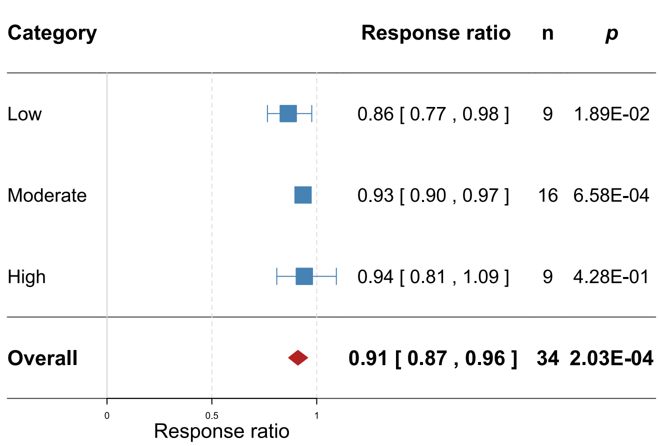

Fig. 5. The effects of plastic concentrations on methane production. The blue square symbols show mean effect size with error bars representing 95 % confidence interval, and the red diamond represents the summary effect. A ratio < 1 indicates that the response from the treatment (including plastic) is lower compared to the control group. n refers to sample size, and *p* means the *p*-value of the Q test with $p < 0.05$ indicating a significant difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

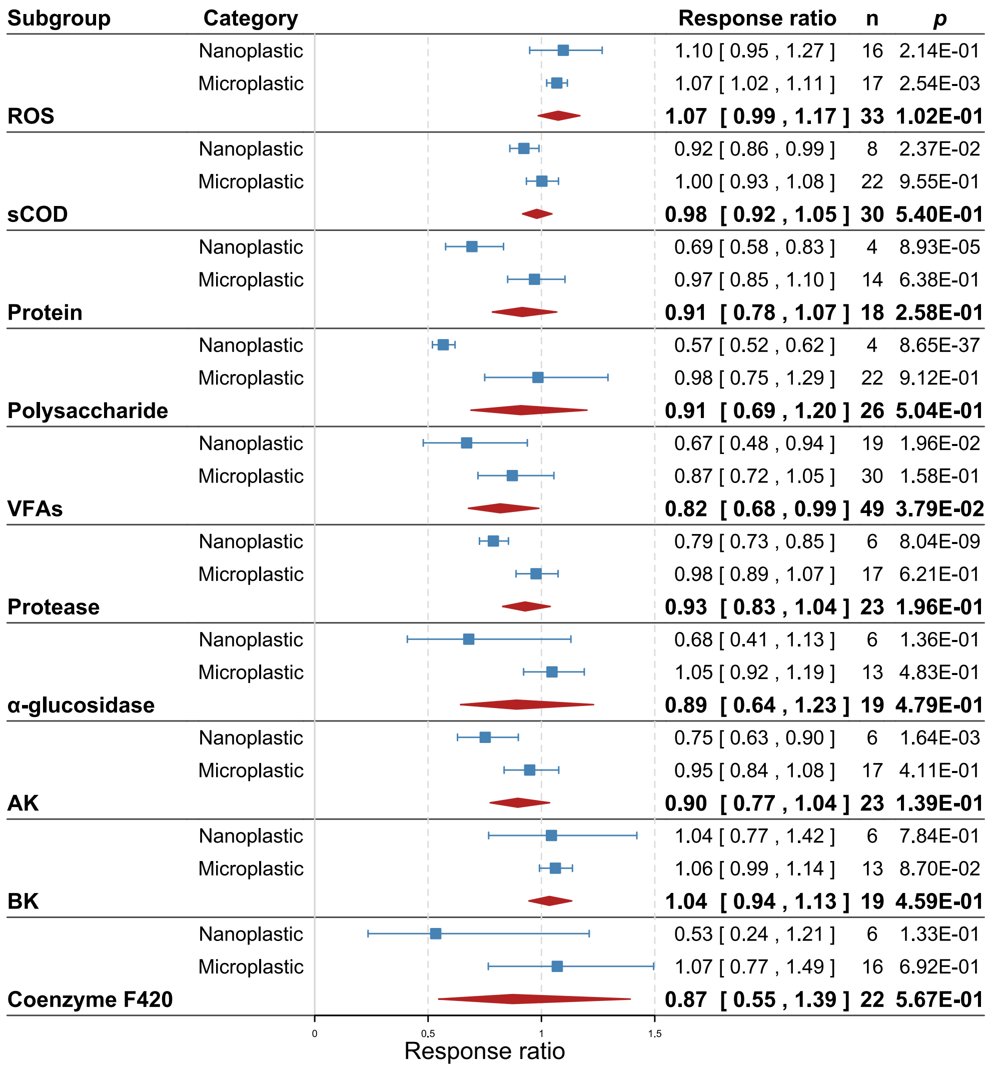

Fig. 6. The effects of plastic particle sizes on various parameters during anaerobic digestion. The blue square symbols show mean effect size with error bars representing 95 % confidence interval, and the red diamond represents the summary effect. A ratio < 1 indicates that the response from the treatment (including plastic) is lower compared to the control group. n refers to sample size, and *p* means the *p*-value of the Q test with $p < 0.05$ indicating a significant difference. ROS: reactive oxygen species; sCOD: soluble chemical oxygen demand; VFAs: volatile fatty acids; AK: acetate kinase; BK: butyrate kinase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the NP category not only showed significant differences in many indicators, but also demonstrated an approximate 10–30 % increase in the impact on each parameter compared to the MP category (Fig. 6). These results corroborated several studies, Zhang et al. revealed that PS NPs had higher inhibition capacity on methane production than PS MPs [43]; the same result was evidenced in another research, where 1 μm PS particles were more inhibitory to AD than 0.1 and 1 mm [10]. Although large size plastics are less hazardous for AD, they could be subjected to mechanical forces in AD system, such as mixing, impaction, and compression, which cause them to break into smaller fragments.

3.4. Toxicity mechanism of plastic pollution on methane production

The pairwise correlations results were shown in Fig. S7, indicating that plastic particle size, sCOD, and VFA were linked with methane production. The high correlation shown between the enzyme activities may be attributed to the fact that the data in this segment originated from the same few articles [24,10,51,23]. The causal relationships among plastics, ROS, sCOD, VFA, enzymes, and methane production were explored by SEM (Fig. 7). The latent variable, plastics, had significant influence on ROS ($\lambda = 0.598$, $P < 0.05$), sCOD ($\lambda = -0.933$, $P < 0.05$), and methane production ($\lambda = -0.574$, $P < 0.05$). In addition, the following pathways were also significant, including ROS to enzymes activities ($\lambda = -0.452$), sCOD to VFA ($\lambda = 0.832$), sCOD to enzymes activities ($\lambda = -0.481$), and VFA to methane production ($\lambda = 0.136$). In summary, the results demonstrated that plastic pollution in AD system affected methane production through two main pathways, inhibiting the solubilisation of organic substances (the primary pathway) and the induction of ROS production. Thereafter, less organic substances and higher ROS stress led to a decrease in the activity of key enzymes from bacterial and consequently a reduction in the production of VFA, which ultimately resulted in a decrease in methane production. Although studies have confirmed that plastics pollution had a positive impact on the solubilisation of sCOD and a negative impact on ROS production, the quantification of these effects was not available [40,49]. This study provided insight into the effects of plastic pollution on methane recovery in AD systems. Future work could focus on the mechanisms by which plastics affect sCOD solubilisation, thereby developing effective approaches to enhance the solubility of complex compounds (e.g. proteins and polysaccharides) in organic wastes.

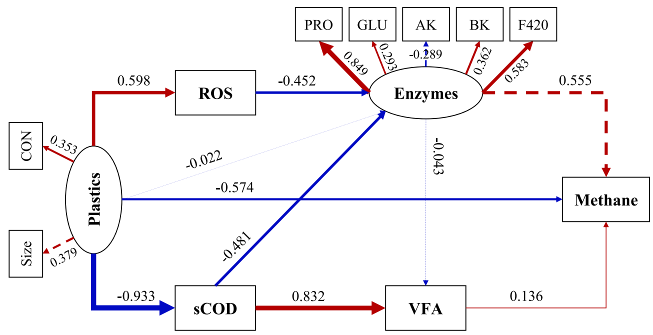

Fig. 7. Structural equation model showing the direct and indirect effects of plastic pollution on the pattern of potential methane production. The red and blue arrows show positive and negative relationships, respectively. Solid arrows indicate significant effect sizes ($p < 0.05$, dashed lines $p > 0.05$), where the thickness of arrows represents the strength of the relationship. The hypothetical model shows good fitness by $\chi^2 = 9.072$, $P = 0.826$, GFI = 0.947 and RMSEA < 0.05). CON: concentration; ROS: reactive oxygen species; sCOD: soluble chemical oxygen demand; VFA: volatile fatty acids; PRO: protease; GLU: α-glucosidase; AK: acetate kinase; BK: butyrate kinase; F420: coenzyme F420. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Limitations of this research

The results of this study have several limitations due to the quality and quantity of data collected from publications. Although the predefined criteria for data inclusion and exclusion can help to ensure that this study is targeted and rigorous, they could exclude potentially related publications that do not meet these criteria and may restrict the generalisability of the findings in this study. Moreover, the data distribution of some features was inconsistent owing to a variety of variations in experimental goals, methodologies, and conditions. For example, the plastic content in the environment was often measured by counting, i.e. the number of plastic particles identified in a specific mass or volume of the environment [56]. Therefore, the collected data for plastic concentration contained particles/L and the common unit (mg/L) [21,29,43]. In addition, MPs have been receiving a lot of attention from researchers because of their small size and high capacity for transport across environments [57,58]. Studies on macroplastics has been much less intense in comparison, which resulted in relatively few data being collected on macroplastics, with data on some features obtained from only two articles (Fig. 4). This may introduce bias which could cause uncertainty conclusions and misinterpretations. Furthermore, the effect of plastics on AD is a complex issue that depends on multiple factors and cannot be directly and accurately measured. To reduce the complexity of the experiments, single plastics were commonly used as materials to explore the effect mechanisms of plastics on AD. Therefore, it is difficult to systematically evaluate the effect of mixed plastics on AD due to the limited number of publications and data that can be extracted.

This study focused on the impact of plastic pollution on AD and elucidated the fundamental toxicity mechanisms by which plastics affect the methane production. However, methane production is not only related to physicochemical parameters during AD, the microbial composition, especially functional microbes, plays a more important role [10]. Studies found that plastics affected the relative abundance of various anaerobes involved in hydrolysis, acidogenesis and methanogenesis. For example, in the presence of polyamide MPs, the total content of Bacteroidia, Gammaproteobacteria, Clostridia, Anaerolineae and Aminicenantia in anaerobic digesters decreased from 58.6 % to 46.2 %, and the majority of these microorganisms play a significant role in hydrolysis and acidification processes [53]; Both *Methanomassiliicoccus* (a hydrogen-dependent methanogens) and *Methanothrix* (decarboxylating acetate to methane) were reduced in the bioreactors with PS NPs, indicating that both pathways for methanogenesis were inhibited when exposed to PS NPs [29]; PVC MPs with 60 particles/g of TS significantly reduced the abundant of various anaerobes associated with hydrolysis (e.g., *Rhodobacter* sp.), acidogenesis (e.g., *Garciella* sp. And *Proteiniborus* sp.), and methanogenesis (e.g., *Methanosaeta* sp.) [20]. In these studies, the stacked histograms were used to present the microbial relative content, from which accurate data was difficult to extract. And the microbial community fluctuated greatly among different experimental conditions and AD system [59,60]. Hence, future research should focus on the construction of a comprehensive database that includes studies with microbiome data under uniform experimental conditions and similar experimental methodologies. In addition, plastics as organic matter undergo a series of dynamic biochemical processes during AD [61]. If more efforts to characterise the plastics involved in AD process can be carried out, more comprehensive descriptions of the toxicity mechanisms may be achieved. In the current dataset, there were only three types of plastic features (including plastic type, particle size, and concentration) that can be extracted from the publications. With the development and availability of microscopic characterization tools, additional categories of plastic variables involved in AD (like key functional groups, contact area with microbes, location in the microbes, or microbial uptake concentration) will be identified and discussed.

4. Conclusions

Despite the relatively low quantity of studies on the influence of plastics pollution on methane production in AD, this study attempted to obtain a comprehensive perspective on the mechanisms of the effects. The *meta*-analysis revealed that the plastic types, plastic particle size, and concentration significantly influenced methane production potential. Both MPs and NPs equally influenced ROS, sCOD, VFA, and various key enzyme activities. However, the influence of NPs was greater in terms of these physiological and biochemical parameters during the AD process, as emphasised by most of the studies. The existence of plastics inhibited the dissolution of organic substances and induced ROS generation, which was unfavourable for the AD performance. Moreover, we have revealed the quantitative relationship between plastic pollution and methane production, which provided a scientific basis for energy recovery from organic wastes containing plastics on a global scale.

CRediT authorship contribution statement

Zhenghui Gao: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Hang Qian:** Writing – review & editing, Visualization, Methodology. **Tianyi Cui:** Writing – review & editing, Investigation. **Zongqiang Ren:** Writing – review & editing. **Xingjie Wang:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2024.149703.

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