

*Original Research*

# Pumice as Biological Carriers Improve Impact Load Resistance of UASB Reactors During the Treatment of Raw Incineration Leachates

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## Abstract

In the present research, the effects of pumice on impact load resistance and microbial community structure in anaerobic granular sludge of up-flow anaerobic sludge bed reactors (UASB) were studied. Our results indicated that the addition of pumice was able to promote the degradation of organic matter, reduce the start-up time, and improve the impact load resistance of the UASB reactor. Compared with the control, the start-up time of the UASB reactor was reduced by 12 days. When the organic load gradually increased from 9.84 kg COD/(m<sup>3</sup>·d) to 33.28 kg COD/(m<sup>3</sup>·d), the average COD removal rate in the reactor containing pumices increased by 10.53% as compared to control. High-throughput sequencing showed that the addition of pumices provided a favorable environment for microbial aggregation, and increased the richness and diversity of microorganisms in the granular sludge. The community structure of methanogens indicated that, in the presence of pumices and under the impact of organic load, the dominant methanogens were Methanosaeta instead of Methanobacterium.

**Keywords:** pumice, impact resistance, microbial community structure, UASB

## Introduction

Leachate produced in municipal solid waste (MSW) incineration is a kind of complex and highly concentrated fluid. It generates in the garbage storage pit stacking step during the anaerobic acidification of the organic components present in the MSW [1].

Landfill leachate usually contains a wide variety of organic pollutants, as well as high concentrations of salts, heavy metals, ammonia nitrogen, and total nitrogen. In different places, the leachate varies greatly due to the diversity of garbage components. The improper treatment of leachate would largely pollute the environment. Therefore, developing a suitable treatment method during leachate is still a big challenge [2].

UASB reactor is an effective treatment method for waste incineration generated leachate. Recently, many studies have been focused on the UASB reactor based

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leachate treatment [3]. However, some aspects limit the practical application of UASB reactors, such as low anti-impact load and long starting time [4]. Thus, in order to improve the treatment efficient of the UASB reactor on leachate, it is an urgent need to improve anti-impact load performance and shorten the start-up time of UASB reactors, in order to provide proper and efficient leachate treatments.

In order to solve those problems, a lot of studies have been conducted on improving the reactor stability and shortening the starting time. Former reports have indicated that nucleation carriers can effectively improve the performance of UASB reactors. These carriers can accelerate the microbial aggregation, shorten the formation time of granular sludge, and enhance the stability of the anaerobic system in general [5]. Chen et al. studied the impact of biocarbon on the performance of aerobic granulation and reactor during oil wastewater treatment. It was found that adding biocarbon can shorten the aerobic granulation time and increase the ability to resist the impact load [6]. Lei and Xu et al. using the activated carbon as the carrier of the UASB reactor, they found that activated carbon can shorten the starting time, increase methane production, and enhance the resistance of anaerobic granular sludge to organic load impact [7, 8]. The above studies show that adding carrier material is an effective method to improve the performance of UASB reactor. It is also found that honeycomb stone can be used as artificial fillers for the treatment of sewage wastewater and carrier of microorganisms [9, 10], however, few studies use pumice as a carrier material for UASB reactors. As a natural green carrier, pumice displays excellent characteristics of high porosity, light weight, high strength, and corrosion resistance against acid and alkali [11]. Compared with other carrier materials, pumice has better microbial compatibility and economic and environmental protection. Thus, the objective of the present study is to determine whether the introduction of the pumice into the UASB reactor can improve the anti-impact load capability and shorter start-up times in leachate treatment. Particularly, our study uses the actual waste leachate as the treatment object, without artificial water distribution, which makes the experimental operation conditions more in line with the practical engineering applications.

## Material and Methods

### Experimental Devices

The experimental device (shown in Fig. 1) consisted of two plexiglass cylindrical UASB reactors (4.5 L and 2 L), a peristaltic pump, a heating rod and a circulating pump. Dimensions were 750, 89, and 110 mm in height, inner diameter, and outer diameter, respectively. The temperature was maintained at  $35\pm 1^\circ\text{C}$  and controlled by the interlayer water bath present between the inner

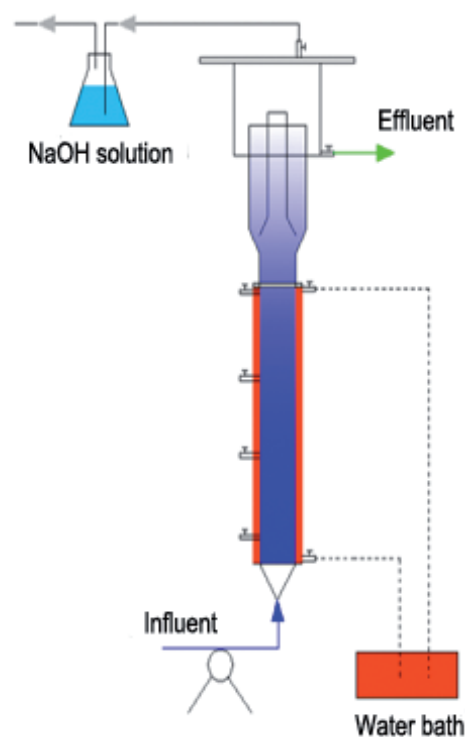


Fig. 1. Schematic diagram of the UASB reactors.

layer and outer layer of the reactor. The influent flow rate was controlled using a peristaltic pump. The reactor reaction area was wrapped with shading paper to avoid the influence of light on microorganisms.

### Inoculated Sludge and Fresh Leachate

A reactor was used as an experimental control group (R1). The amount of inoculated sludge was about 30% of the effective volume of the reactor. The other reactor, 500 g pumice were added, and labeled as the experimental group (R2).

The sludge was collected from the sludge thickening tank of a landfill leachate power plant located in Anhui Province, China. The ratio of volatile (VSS) to total suspended solids (VSS/TSS) in the sludge was 0.57. The fresh leachate was collected from the UASB anaerobic tank of the power plant as the feed water (shown in Table 1). The pH of the influent was adjusted at  $7.0\pm 0.3$  by adding sodium bicarbonate. The HRT (hydraulic retention time) was 15 hours during the whole operation.

### Analytical Methods

The chemical oxygen demand (COD), TSS and VSS were measured according to the standard procedures [12]. DOMs concentrations of incoming and outgoing water samples were analyzed using a F-7000 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength and emission wavelength range of the 3D fluorescence spectra were 200 nm~450 nm and 280 nm~550 nm, respectively; the slit width was

Table 1. The physicochemical parameters of leachate at MSW incineration plant located in Hefei, China.

Parameters	MSW incineration leachate (mg/L)
COD	67445~71233
NH <sub>3</sub> -N	2970~3154
TN	3542~3820
TP	101~112
pH	5.2~6.5

set to 5 nm, the scanning speed was 2400 nm/min [13]. Because of the large fluorescence intensity of the diluted leachate, water samples were filtered with 0.45 nm filter membranes before analyses. In order to reduce the internal filtration effect produced by the fluorescence measurement, the filtered water samples were diluted. In order to eliminate the effect of Rayleigh scattering, a 290 nm cut-off filter was installed on the emitting fluorescence side. In addition, in order to eliminate the influence of Raman scattering, the MATLAB software was used to draw the 3D fluorescence spectrum map, and the ultrapure water blank data was subtracted from the water sample data [14].

### Microbial Community Analysis

The sludge samples were collected from the control group reactor (R1) and the experimental group reactor (R2) at days 100 (stable stage) and 162 (strengthen stage), respectively. Microbial DNA extraction was carried out following the methods provided by the guidebook included in the soil DNA kit (purchased from Omega Bio-tek, Inc.) The quality of the extracted DNA was determined using 2% agarose gel electrophoresis. The V3+V4 regions of 16S rRNA were amplified with the bacterial primers 338F: 5'-ACTCCTACGGGAGGCAGCA-3' and 806: 5'-GGACTACHVGGGTWTCTAAT-3'; for

methanogen, MLf5'-GGTGGTGMGGATTACACARATAYGCWACAGC and MLr5'-TTCATTGCRTAGTTWGGRTAGTT [15, 16]. The amplicons were delivered to Illumina Miseq platform (Biomarker Technologies, Beijing) for high-throughput sequencing.

## Results and Discussion

### Effect of Pumice on Impact Load Resistance

As shown in Fig. 2, during the 168 days of anaerobic process in the UASB reactors, three stages are present: start-up stage, stable stage and strengthen stage. The COD concentrations of the influents are in the ranges of 950~2500, 2500~5000, and 5000~18000 ppm at the start-up, stable, and the strengthen stage, respectively. During the start-up stage, regardless pumice are present or not in the UASB reactors, the COD concentrations gradually increased from 950 to 2500 mg/L. As the reactor continued continuously, the COD removal rates are stably over 85%, R1 reaches stability at day 15 (pumice added), while R2 (control reactor) at day 27.

At the stable stage, the COD influent loading maintains at medium level. In this case, the concentrations slowly increase from 2500 to 5000 mg/L. This result indicates that both reactors can efficiently remove COD, and the COD removal efficiencies of those two reactor are both more than 90%. With regards to stability, reactor R2 shows smaller fluctuations with the influent than R1.

Fig. 3 displays the detailed daily treatment capacity and COD removal efficiency of at the strengthen stage of the UASB reactors. When the organic loading rates increase from 13.85 to 33.28 kg COD/(m<sup>3</sup>·d), the COD removal efficiencies in R1 and R2 decrease. With the increase of influent organic loads, the COD removal rate of the reactor R2 returns to 96% after 12 days and remained stable afterwards. These results further demonstrate that the addition of pumice

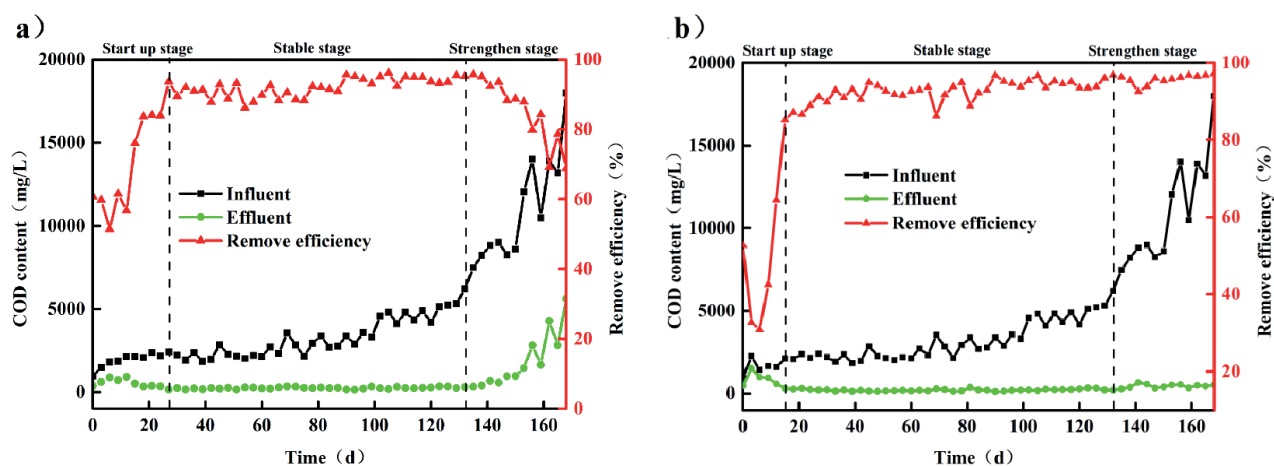


Fig. 2. The removal efficiencies of COD during different stages in R1 a) and R2 b) reactors.

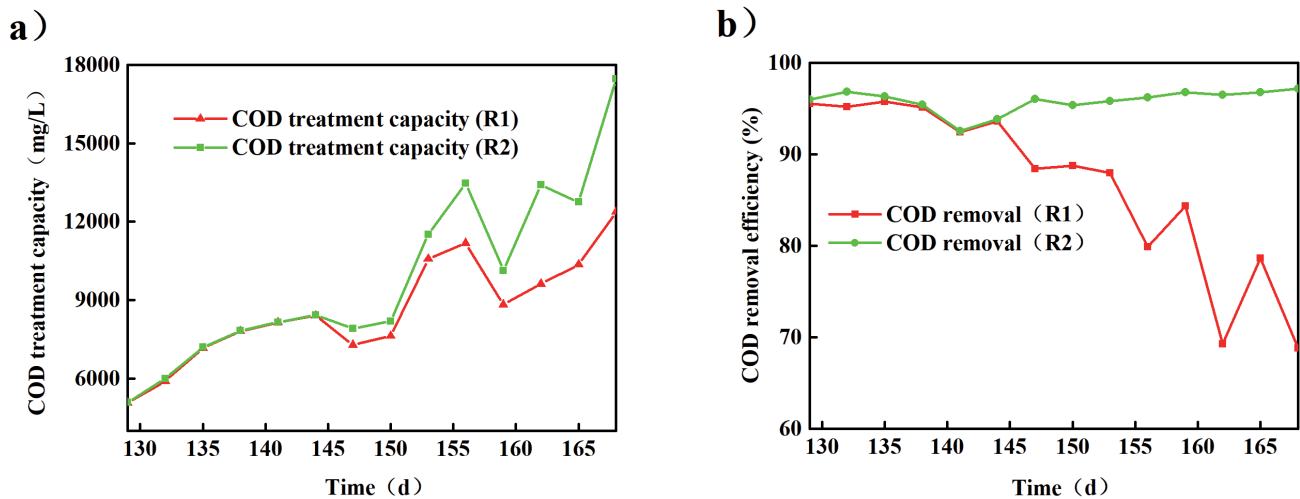


Fig. 3. a) The COD daily treatment capacity of R1 and R2 reactors at the strengthen stage, b) The COD removal rate of R1 and R2 reactors at the strengthen stage

is the UASB reactor effectively improves the performance during incineration leachate treatment, even when the OLR reached levels as high as 33.3 kg COD/(m<sup>3</sup>·d). In contrast, the COD removal efficiency in R1 is unstable, which decreases from 95.5% to 68.8% when OLR increased from 9.8 kg COD/(m<sup>3</sup>·d) to 33.3 kg COD/(m<sup>3</sup>·d) (strengthen stage).

The addition of pumice enhanced COD removal as well as resistance to shock loadings. Furthermore, the start-up time of the UASB reactor is also reduced. Thus, the pumice contained reactor exhibits the best performance. The enhanced activities may be attributed to the development of a diverse microbial community. The high specific surface area and total pore volume of the pumice provide a good habitat for microorganisms. The presence of pumice help increasing microbial abundance, improve microbial activity, and promote the degradation of soluble organic matter.

### Three-Dimensional Fluorescence Analysis of Incoming and Outgoing Water

EPS plays an important role in the physical and chemical properties of anaerobic granular sludge. SMP is composed of EPS hydrolysates and decay products of active cells, which are the main components of soluble organic matter in wastewater [17]. In the 3D-EEM spectra, five clear peaks can be observed. They are corresponding to five different components: (a) peak A, Ex/Em = 225/320 nm corresponds to the tyrosine protein; (b) peak B Ex/Em = 225/340~360 nm corresponds to tryptophan class proteins; (c) peak C Ex/Em = 280/350(305) nm indicates the soluble microbial metabolites; (d) peak D Ex/Em = 330/410(415) nm indicates the presence of humic acid substances; and peak E Ex/Em = 250/450(455) nm corresponded to ferric acid [18]. Fig. 4 shows the changes of the fluorescence peaks of the landfill leachate before and

after treatment with the UASB reactor. After treatment, the fluorescence intensity A, B, and C significantly decrease, and the absorption peak which correspond to tyrosine protein-related substances disappear. This result indicates the presence of fulvic acids in the effluent. It is likely that these fulvic acids were produced by microbial metabolism [19]. Comparing these results to that of control (R1), the peak intensities of B, C, D, and E in R2 are 10.5%, 14.8%, 9.9%, and 22.9% lower, respectively. These results indicate that the addition of pumice improves the degradation of soluble organic matter and promoted the metabolism of microorganisms. However, pumice does not have significant effect on SPM components.

### Bacterial Community Diversity Analysis

Sludge samples from Day 100 (R1(100) and R2(100)) and day 162 (R1(168) and R2(168)) were sequenced using the Illumina Miseq platform. The alpha diversity indexes are displayed in Table 2. In all samples, coverage are higher than 99%, indicate that the sequencing results represent the real composition of the samples [20]. The presence of pumices in the reactor significantly increases the Shannon index on the days of 100 and 162. In contrast, Simpson diversity is significantly reducing in the presence of pumice on the days of 100 and 162. At the same time, the Chao index and the ACE index increase, indicating that the pumice positively affects the development and richness of bacterial diversity.

Fig. 5 displays the results of the statistical analysis based on OTUs (operational taxonomic units). As the results shown, a total of 551 OTUs are obtained on day 100 after sequencing. In addition, 501 species are present in all samples, and the species unique to R1 and R2 are 17 and 33, respectively. Moreover, on day 162, total OTUs increases, and the number of



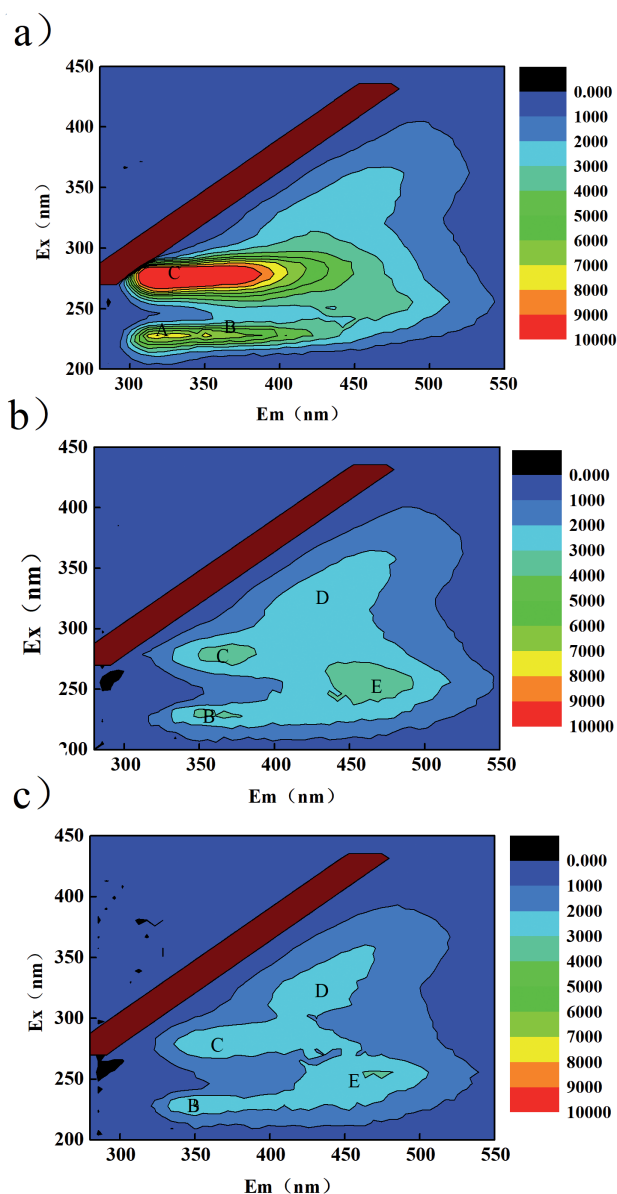


Fig. 4. a) The three-dimensional fluorescence spectra of R1 and R2 reactor influent water at the strengthen stage, b) and c) The three-dimensional fluorescence spectra of R1 and R2 reactor effluent water at the strengthen stage, respectively.

endemic species in R1 and R2 increases to 43 and 71, respectively. At the same stage, the number of OTUs endemic species in reactor R2 is higher than that in reactor R1. All these data suggest that the addition of

pumice increases the species and number of bacterial OTUs. In consequence, these materials may favor the degradation of some characteristic pollutants.

### Changes in Microbial Community Structure

Fig. 6 presents the distribution of bacteria at the phylum, class, and genus levels in the anaerobic granular sludge. According to the results (Fig. 6a), in both reactors, the dominant phyla in the granular sludge are Firmicutes, Proteobacteria, Bacteroidetes, Chloroflexi, and Synergistetes. Thus, the presence of pumice does not affect the number of species; however, they affect their abundance. On days 100 and 162, the relative abundance of Proteobacteria and Chloroflexi in the R2 reactor are always higher than that in R1. Proteobacteria are able to degrade complex macromolecular organic matter and long-chain VFAs [21]. Chloroflexi are usually found in the anaerobic digestion system. These fermentative bacteria produce hydrolases that are able to degrade soluble microbial products, including soluble proteins and soluble polysaccharides [22]. Other studies have shown that Chloroflexi displays the ability to transfer extracellular electrons to electron receptors, which can promote DIET, improving the anaerobic digestion efficiency of reactors [23]. Moreover, after organic load, the relative abundance of Firmicutes in the R2 reactor increases from 27.7% to 49.0%, while that in R1 is relatively stable. Firmicutes are widely found in the anaerobic digestive system. They always contain endospores that resist harsh environments and are the main bacteria responsible for removing COD [24].

At the class level (Fig. 6b), it is found that different reactors displayed different proportions of dominant species (Fig. 5b). On days 100 and 162, the predominant microbes are Clostridia, Bacteroidia, Deltaproteobacteria, and Anaerolineae. After the organic influent loaded, the relative abundance of Clostridia in the R2 reactor increases from 23.1% to 40.3%. However, the increase is relatively small in the R1 reactor. Clostridia are strictly anaerobic bacteria that produce butyric acid and acetic acid by decomposing carbohydrates [25]. This process promotes the growth of related acid-type methanogens and improves the degradation efficiency of organic matter.

At the genus level (Fig. 6c), *Syntrophomonas* accounted for 2.47%, 2.21%, 3.29%, and 3.91% of R1(100), R2(100), R1(162), R2(162) total bacterial genera, respectively. As members of specialized

Table 2. On days 100 and 162, the alpha diversity index of bacterial community in sludge of R1 and R2 reactors.

Type of strain	Stage	Sample	OTU	ACE	Chao1	Simpson	Shannon	Coverage
Bacteria	Stable stage	R1(100)	518	529.7151	532.5882	0.0486	4.1772	0.9993
		R2(100)	534	536.2273	539.0770	0.0180	4.8000	0.9997
	Strengthen stage	R1(162)	508	524.9262	536.8889	0.0238	4.6216	0.9992
		R2(162)	536	543.1494	545.7308	0.0209	4.7303	0.9996

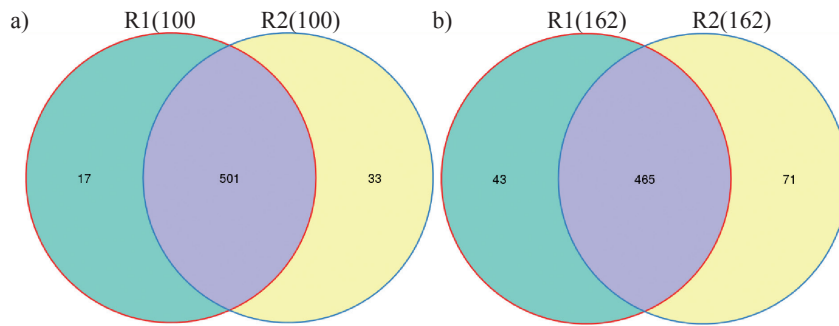


Fig. 5. On days 100 and 162, the quantity of OTU with 97% similarity in anaerobic granular sludge of R1 and R2 reactors.

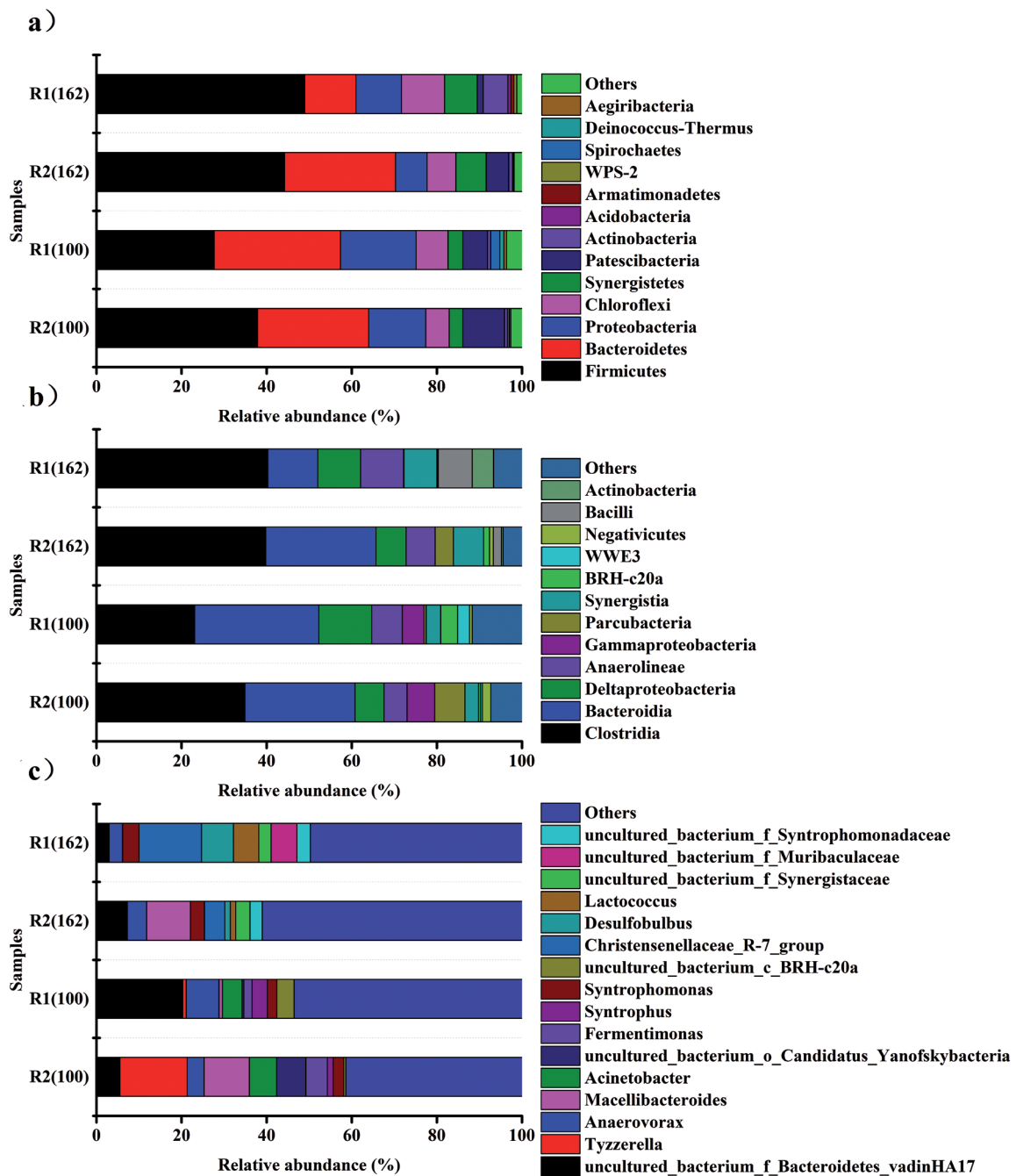


Fig. 6. On days 100 and 162, the bacteria community structure in anaerobic granular sludge of R1 and R2 reactors, a) phylum level; b) class level; and c) genus level. “Others” represents all classified taxa that were <0.1% in all samples.

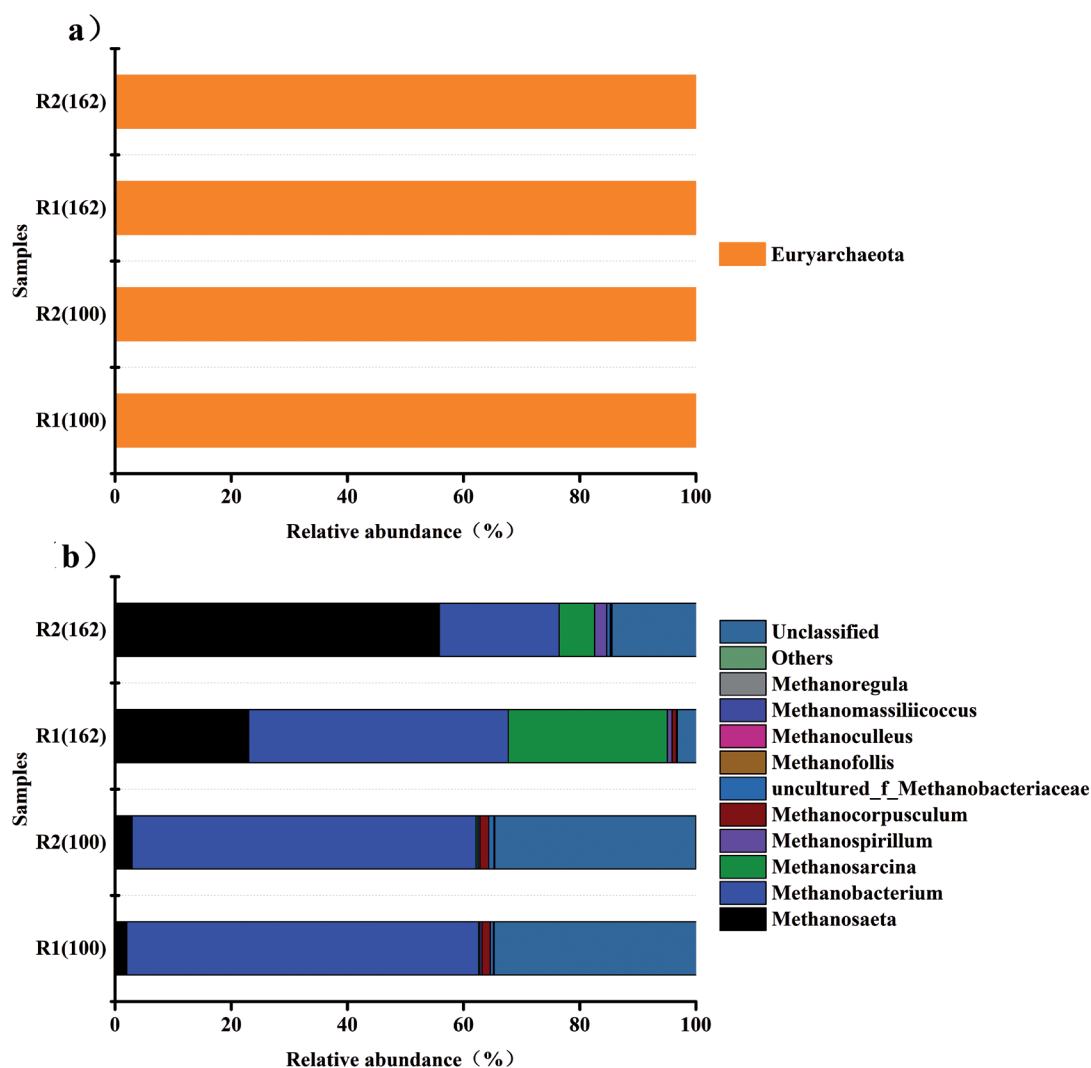


Fig. 7. On days 100 and 162, methanogens community structure in anaerobic granular sludge of R1 and R2 reactors, a) phylum level and b) genus level. "Others" represents all classified taxa that were <0.1% in all samples.

anaerobic and cocultured bacteria, *Syntrophomonas* possesses the ability to oxidize butyrate and other VFAs into acetic acid. They are usually present during hydrogen synthesis in combination with methanogens and other hydrogen-using microorganisms. Their presence in the system, although not very abundant at the onset stage, is likely to induce functional microbial growth and promote digestion processes [26].

#### Changes in Community Structure of Methanogens

As known, methane is produced during the anaerobic degradation of organic matter. Methane production is considered to be the action of related methanogens [27]. At the phylum level, microbial community structure showed that Euryarchaeota is the dominant species in R1 and R2. On day 100, the dominant methanogens in R1 and R2 are both *Methanobacterium*, and their relative abundances account for 60.55% and 59.23%

of the total methanogen sequence, respectively. In addition, *Methanosaeta* account for only 2.01% and 2.91%, correspondingly. On day 162, the relative abundance of *Methanobacterium* in R1 and R2 decrease to 44.71% and 20.59%, respectively. On the other hand, *Methanosaeta* relative abundance increase to 22.98% and 55.86%, correspondingly. The abundance of dominant methanogens also changed.

*Methanobacterium* are the hydrogen-type methanogenic bacteria present during the anaerobic treatment of organic matter. They use  $H_2/CO_2$  or formic acid to produce methane [28]. Also, *Methanosaeta* are strict anaerobes, which not only use acetic acid as substrate to produce methane, but also reduce carbon dioxide to methane by the effect of DIET receiving Electrons [29]. *Methanosarcina* are also acid-type methanogens; however, unlike *Methanosaeta*, they tolerate high salinity and high acetic acid concentrations [30]. With the increase of organic load, *Methanobacterium* in both reactors show different

degrees of inhibition, which promoted the growth of Methanosaeta. The increase in the relative abundance of Methanosaeta in the R2 reactor is 2.6 times higher than that in R1. It is likely that pumices improved the acid buffer capacity of the anaerobic system and maintained a relatively stable environment for the microorganisms. The acid accumulation in the control group increase slowly, inhibiting the growth rate of Methanosaeta and stimulating the growth of the acid-resistant bacteria Methanosarcina.

### Conclusions

The addition of pumice during anaerobic processes represents a feasible technology to improve pollutant removal and anti-shock loading. Even when the organic loading rates increased to 33.28 kg COD/(m<sup>3</sup>·d), the COD removal efficiency remains stable at >90%. However, the maximum organic loading rates of the reactor without pumice is only 13.85 kg COD/(m<sup>3</sup>·d). Further investigation of the microbial community indicates that pumice can increase the bacterial abundance and diversity, promote the growth of Proteobacteria and Chloroflexi. With the increase of organic loading rates, pumice are favorable for the growth of Methanosaeta and enhanced the UASB acid buffer capacity. These results verify the effectiveness of the application of pumice in UASB reactors and provide a good theoretical basis for the treatment of landfill leachate.

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### Conflict of 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.

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