


## Article

# Biogas Production and Fundamental Mass Transfer Mechanism in Anaerobic Granular Sludge

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**Abstract:** Anaerobic granules are responsible for organic degradation and biogas production in a reactor. The biogas production is entirely dependent on a mass transfer mechanism, but so far, the fundamental understanding remains poor due to the covered surface of the reactor. The study aimed at investigating the fundamental mass transfer characteristics of single anaerobic granules of different sizes using microscopic imaging and analytical monitoring under single and different organic loadings. The experiment was conducted in a micro reactor and mass transfer was calculated using modified Fick's law. Scanning electron microscopy was applied to observe biogas production zones in the granule, and a lab-scale microscope equipped with a camera revealed the biogas bubble detachment process in the micro reactor for the first time. In this experiment, the granule size was 1.32, 1.47, and 1.75 mm, but 1.75 mm granules were chosen for further investigation due to their large size. The results revealed that biogas production rates for 1.75 mm granules at initial Chemical Oxygen Demand (COD) 586, 1700, and 6700 mg/L were 0.0108, 0.0236, and 0.1007 m<sup>3</sup>/kg COD, respectively; whereas the mass transfer rates were calculated as  $1.83 \times 10^{-12}$ ,  $5.30 \times 10^{-12}$ , and  $2.08 \times 10^{-11}$  mg/s. It was concluded that higher organic loading and large granules enhance the mass transfer inside the reactor. Thus, large granules should be preferred in the granule-based reactor to enhance biogas production.

**Keywords:** micro reactor; granular sludge; mass transfer; microscopy; bubble production

## 1. Introduction

Energy is the driving force for the socioeconomic development of a country. Since the early 1970s, the energy demand in domestic and industrial sectors has increased which resulted in the depletion of the fossil fuel. Therefore, access to affordable and sustainable energy has become a global challenge [1]. The substitutes for fossil fuel depletion are renewable energy resources such as biogas, solar, wind energy, etc. In recent years, an integrated biogas production and conversion process for sustainable bioenergy has been proposed and analyzed [2]. It is noteworthy that biogas production via anaerobic digestion proves to be a cheap, environment-friendly, and sustainable energy source [3–5]. Vietnam, Brazil, China, India, Nepal, and some African countries have invested in indigenous biogas technology where granule-based anaerobic reactors remain highly efficient in producing a large volume of biogas [6]. The anaerobic bacteria in the granular sludge removes organics and is one of the most important components of the bio-reactor. At present, granule-based reactors include upflow anaerobic sludge blanket (UASB), internal circulation (IC), and expanded granular sludge blanket (EGSB) [7,8]. In developing countries, the decentralized approach has been adopted where small-scale biogas

plants are utilized to transform waste into gas through anaerobic fermentation of organic materials [9]. The reason being that the most commonly available feedstock material is animal dung or human excreta. Such type of waste is a challenge for rural waste management [10,11].

The benefits of aerobic and anaerobic granules applied in wastewater treatment and biogas production have been widely acknowledged [12]. Microbial granules usually showed a relatively higher organic removal rate (e.g., nitrogen removal rate (NRR) of 74.3–76.7 kgNm<sup>-3</sup>d<sup>-1</sup> in a laboratory-scale upflow anaerobic sludge blanket (UASB)) than anammox flocs as reported by [13]. Furthermore, stable anaerobic granulation and organic removal can be achieved at a higher salinity level as high as 20 g Na<sup>+</sup>/L [14]. Tsui et al. [15] inferred that the gas sparging technique helped accelerate the mass transfer in the reactor along with development of the granular diameter. Municipal wastewater was treated in a microbial sludge reactor and ammonium and total nitrogen removal efficiencies averaged 92.35% and 90.41%, respectively as reported by [16]. Nevertheless, mass transfer resistance in the granules is one of the major factors which alters the reactor performance. The mass transfer values play an important role in modeling studies relating to granular sludge and biofilms in the reactor [17–19].

However, the performance of the anaerobic reactor is not the same when scaled up. A lab-scale experiment will have different results in a pilot scale when compared with a full-scale bio-reactor due to changes in mass transfer condition. The mass transfer condition of the granule is one of the most important factors for stable performance of the reactor [20]. The challenge is that mass transfer phenomena of anaerobic granules is not fully understood because the bio-reactor is fully covered for heat insulation; thus, a reactor is a “black box” and cannot be studied with the naked eye [21]. Several factors contribute to the variation of mass transfer. One important factor is the variable size of granules inside the reactor where the granules’ morphologies are different from each other. Shi et al. [20] reported that the size of the anaerobic granule has an impact on mass transfer; therefore, such difference in morphology leads to dissimilar reactor performance. Other factors such as size of anaerobic granule, inhibitory metal concentration, microbial consortium, pore size, structure, and fluid dynamic environment, etc. influence the mass transfer and biogas production [22–24]. Apart from operational factors, the microbiology of the granule is critically important to understand mass transfer mechanism inside the granule. Rod shaped, round, and flake structured microbial populations in anaerobic granules can influence the mass transfer mechanism [25].

At present there are very few studies with little experimental data about mass transfer in granular sludge [26]. Gonzalez-Gil et al. [27] reported that thick biofilms can limit the mass transfer, resulting in an overall limitation of reactor capacity. Therefore, the influx of substrate and/or out flux of products may become the rate-determining step. Mass transfer limitations are particularly important at low substrate concentrations [20]. The cumulative impact of morphology, microbiology, and operational parameters actually affects the mass transfer condition inside the reactor, which hinders or accelerates the biogas production. The challenge is that mass transfer has not been fully investigated due to complex factors involved in the process as well as limitation of technology [21]. Del Nery et al. [28] reported that efficiency of a granule-based reactor is assessed by examining the physicochemical changes in the influent and the effluent. However, this simple monitoring cannot explain the fundamental biodegradation mechanisms and mass transfer condition inside the reactor. A knowledge gap exists in understanding the mass transfer mechanism and its relationship with selecting the appropriate granule size for better performance of the reactor.

This novel study aimed at investigating the fundamental mass transfer characteristics of single anaerobic granules of different size. The morphology was studied using microscopic imaging and analytical monitoring of molecular diffusion in each granule was carried out in a micro reactor under different organic loadings. The study is of practical importance for researchers and engineers to understand mass transfer mechanism in granules and upgrade granule-based anaerobic reactors.

## 2. Materials and Methods

### 2.1. Morphology of the Granule

The aim of this test was to observe the heterogeneity of anaerobic granules taken from a bulk sample. The spherical granules were separated for the micro reactor experiment. The test was conducted under a microscope that was placed in a customized incubator made of plexiglass (PMMA) to keep the temperature at  $35 \pm 2$  °C for anaerobic biogas reaction in the micro-reactor, as shown in Figure 1. The dimension of incubator box was length 400 mm, width 400 mm, and 600 mm height, respectively. A digital temperature control device and an incandescent bulb were installed for heating the incubator along with an axial fan at the top to keep uniform temperature inside the incubator. The micro-reactor was placed under the microscope equipped with a digital camera and the camera was programmed to take pictures continuously during the experiment. Furthermore, to maintain a humid condition, an appropriate amount of water was placed in a box. The microscope (Motic Group, Fujian Sheng, China) was equipped with a digital camera under the six-fold objective. The diameter was measured with a quantitative image analysis program (Motic Images Advanced 3.2, China).



Figure 1. Incubator for micro reactor.

A scanning electron microscope (FEI, QUANTA 200, Hillsboro, OR, USA) was used to study the channel system of granules. The granules were prepared following the same pretreatment method mentioned in [29]. COD was measured with the COD meter (Hebei Huatong Co., YL-1A, Tangshan, Hebei, China). Volatile suspended solids (VSS) were measured with the weight methods.

### 2.2. Micro Reactor Experiment for Biogas Production

The aim of this test was to achieve an anaerobic environment for the biogas production and bubble formation from a single granule. A special micro reactor was designed for this experiment. The micro reactor for the single granule experiment was also made of plexiglass 50.0 mm long, 25.0 mm wide, and 2.0 mm high as shown in Figure 2. The internal reaction zone was 41.0 mm long, 14.0 mm wide, and 1.0 mm high. The reactor was completely filled with substrate to achieve an anaerobic condition, whereas the experiment was conducted under a static condition.

Anaerobic granules were segregated from bulk sludge obtained from starch wastewater treating full-scale UASB reactor into different sizes. The granules were separated using sieves of the respective size and stored in 500 mL glass bottles. Later, the granules were separately preserved in low strength (COD 200 mg/L) at 35 °C for pre-activation.

In order to assure stable performance, the biogas production of a single granule was measured twice at COD 3000 mg/L. The microscope camera was programmed for 20–30 h and the biogas production was calculated by measuring the volume of biogas bubbles produced in the micro reactor. The shape of bubble was assumed to be spherical due to its small size.

$$V = \frac{\pi h}{3}(R^2 + Rr + r), \quad (1)$$

where  $h$  is height of the reactor,  $R$  is external radius of bubble, and  $r$  is internal radius.



**Figure 2.** Micro reactor for single granule experiment.

### 2.3. Mass Transfer Analysis

Following the micro reactor experiment, an empirical approach was applied to validate the biogas production from a single granule. It is generally understood that molecular and convective diffusion contribute to the mass transfer process in the aerobic as well as anaerobic granule [29,30]. Hydrodynamic conditions were the decisive factors for diffusion process to take place. In the absence of external liquid flow, molecular diffusion is the predominant mechanism. In this study, only molecular diffusion was examined to gain fundamental knowledge inside the granule. A mathematical model was applied with following assumptions.

1. The selected granules were spherical in shape. This was also verified under microscopic experiment.
2. The granules had a homogeneous biofilm of uniform thickness.
3. The synthetic feed was entirely mixed and had uniform concentration throughout the reactor.
4. The mass transfer within the granules would be the rate limiting step rather than external mass transfer. As the granules were porous, the pore network within granule facilitated the mass transfer occurring in the liquid–liquid phase. The granules pore size ranged in nanometers, thus, substrate in molecular level can reach the granule core [27].
5. The substrate solution during the experiment was immobile so molecular diffusion was the primary factor responsible for the mass transfer. The advective transport cannot be neglected but in this study due to very low fluid flow (flow caused when bubbles detached from the granule) its role was not significant [12].

The molecular diffusion ( $F_{MD}$ ) within a granule was calculated in a previous study [29] using the modified Fick's law as follows:

$$F_{MD} = 4 \times \pi \times \left(\frac{d}{2}\right)^2 \times D_M \times C \times \frac{\varepsilon}{\phi}, \quad (2)$$

where  $d/2$  was the radius of the granule (cm),  $D_M$  was the diffusivity of the substrates in water, and  $C$  was the substrate concentration (mg/L).  $D_M$  of glucose was  $0.94 \times 10^{-5}$  cm<sup>2</sup>/s [31].

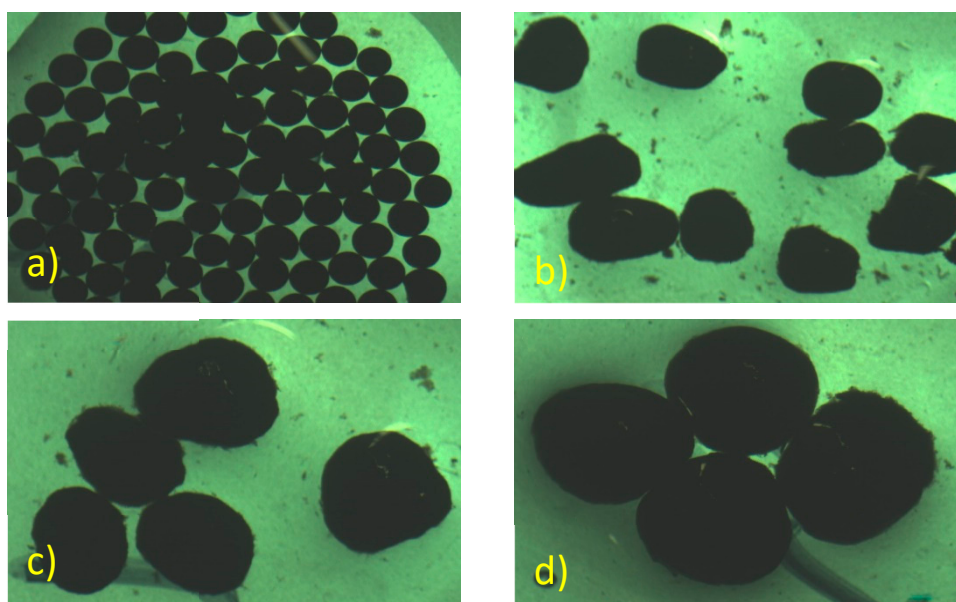
### 3. Results and Discussion

The anaerobic granule characteristics and mass transfer conditions in a micro reactor were analyzed using multiple size granules initially, and afterward a single size granule with variable organic concentration was chosen to understand the biogas production process.



### 3.1. Morphology of the Granule

The outlook of the anaerobic granule is important as it indicates the condition of the microbes. Dark granules indicate an anaerobic condition, whereas grey or white color granules show a partial anoxic condition [32]. In this study, the physical appearance of anaerobic granules was observed under the microscope as shown in Figure 3. Anaerobic granules were found to be nearly spherical and compact in nature. However, very small granules were loose and had no definite shape features. Different size ranges of granules were observed from the bulk sludge sample. Figure 3 illustrates the segregation stages for the biogas production experiment. A grab sample taken from the bulk sources is shown in Figure 3a. It is noteworthy that most of the granules were round. Figure 3b demonstrates a few elongated granules, which were large in shape but not used in this study due to their defective shape. This enlargement is generally caused by the shear forces inside the reactor [33,34]. Mathematical models related to mass transfer assume that granules were spherical in shape [27]. Figure 3c,d illustrates perfectly round and large granules, which were suitable for the investigation of biogas production and mass transfer analysis. Our microscopic results confirm that granules were spherical in nature and could be used for the mass transfer study. Furthermore, the dark colored granules confirmed the anaerobic condition inside the bulk sample. These results were in agreement with [35].



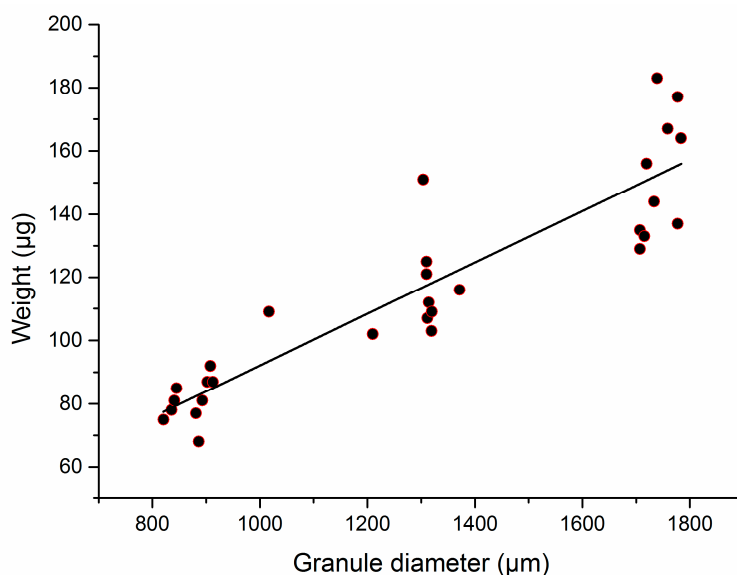
**Figure 3.** Different shapes of granules. (a) bulk sample, (b) elongated granules, (c,d) perfectly spherical granules.

### 3.2. Weight of the Granule

The average weight of the granules ranged between 82, 120, and 170  $\mu\text{g}$ , respectively, whereas the size of the granules ranged between 800 and 1800  $\mu\text{m}$ . On average, 10 granules with size close to the selected granules were chosen for weight measurement. Li et al. [36] reported that granular size plays a pivotal role in development of stratification layers on the granule, which can affect the mass transfer mechanism. The dry weight vs. the size of the granule is plotted in Figure 4. It was observed that the weight of the granule varies for similar-sized granules, revealing a non-homogeneous nature of the granules. The weight of the granule is related with the settling velocity inside the reactor and a large granule settles faster than a smaller granule [37]. During the biogas production process, the size of granules changed due to microbial growth and decay, shear forces, and granule-wall collisions [22]. In this study, a positive but weak relationship ( $R^2 = 0.827$ ) was obtained explaining that the physical appearance of a granule is not enough to judge its performance. Figure 4 shows that the weight

difference is significant for granules; although weight is positively related to granule size, the difference demonstrates that the granules are unique. The empirical equation obtained was as follows:

$$y = 0.0815x + 10.601 \quad R^2 = 0.827. \quad (3)$$



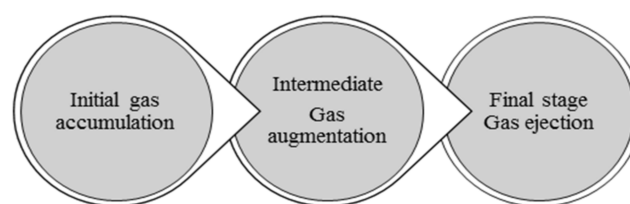
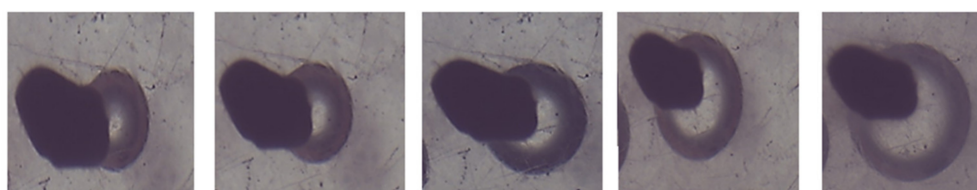
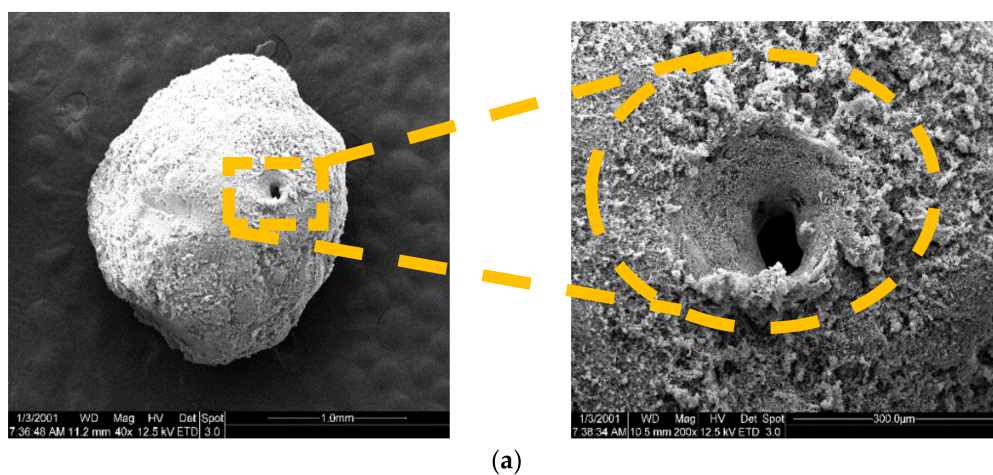
**Figure 4.** Diameter vs. weight of anaerobic granules.

The variation in results provided a basis for the investigation of different-sized anaerobic granules which would lead to choosing the optimal size and weight of granules for a granule-based reactor. Recent studies on anaerobic granules mentioned that granulation in hybrid anaerobic reactors varies due to different microbial consortium thus leading to variation of shape and size in granules [38]. In addition, the weight of the granule represented the spatial dynamics of microbial communities in the granule. Thus, it can be inferred that a heavy granule can have a positive impact on mass transfer due to the availability of a larger microbial population.

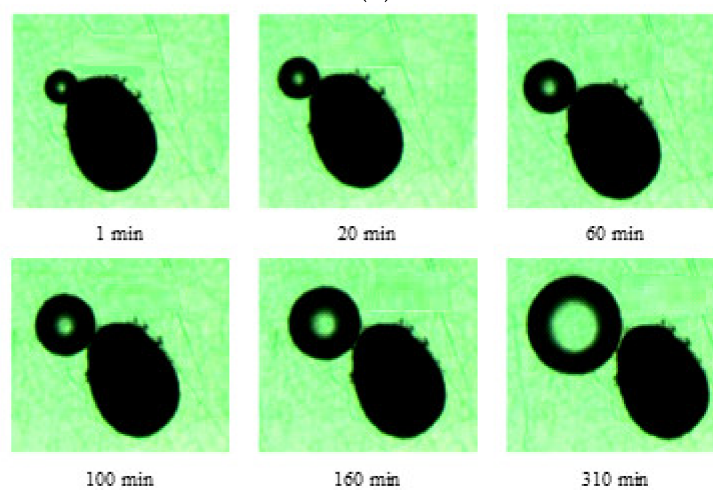
### 3.3. Biogas Production Process in an Anaerobic Granule

The biogas production of individual granular sludge is shown in Figure 5. The results indicate that it is feasible to complete the anaerobic biogas production experiment in the micro reactor as anaerobic conditions had been achieved. First, the substrate was absorbed into the granule through surface pores and subsequently decomposed into biogas. However, the biogas first accumulated inside the small internal pores and finally augmented to escape from the main channel of the granule. The figure indicates the biogas production process and main channel for gas escape. Jian and Shi-yi [39] also studied the internal structure of anaerobic granules and reported that due to internal pressure development there are multiple channels or pathways developed for biogas discharge from the granule.

Figure 5 shows that bubbles are present near the granular sludge during the anaerobic biogas production of particulate sludge and gradually increase with time. The SEM of the granule indicated an opening on the granule surface which points out the pore channel for gas escape. This slow but gradual bubble formation is directly linked to molecular diffusion of the substrate inside the anaerobic granule as molecular diffusion is a time-consuming process and dependent on granule size and distribution [40]. The opening caused by gas pressure in the granule offers a pathway for substrate diffusion and increases the porosity of the granule [41,42].



(b)

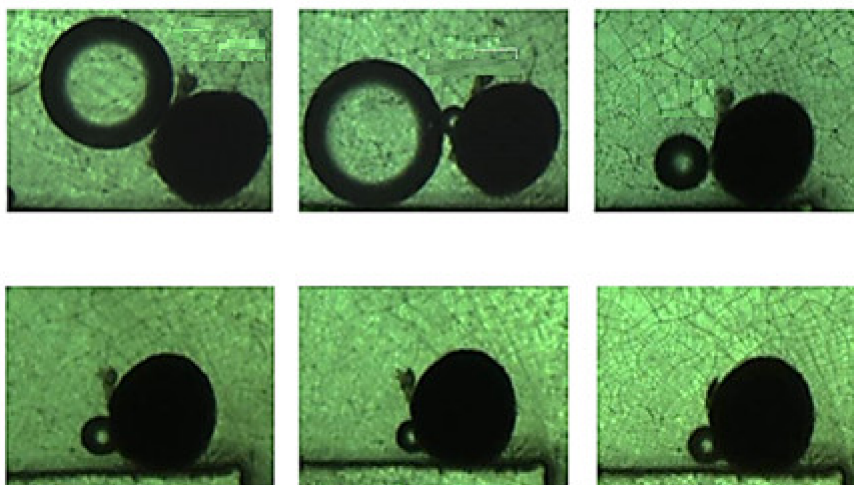


(c)

**Figure 5.** Biogas production process in a single granule.

In most cases, granular sludge produces only one bubble at a time. The location of the bubble is also relatively fixed. During the test, the single granule was tested repeatedly six times, as shown in Figure 6. By comparison, it can be seen that the gas production position of the sludge is basically fixed and is consistent with the finding of the pores with the fixed position on the surface of the

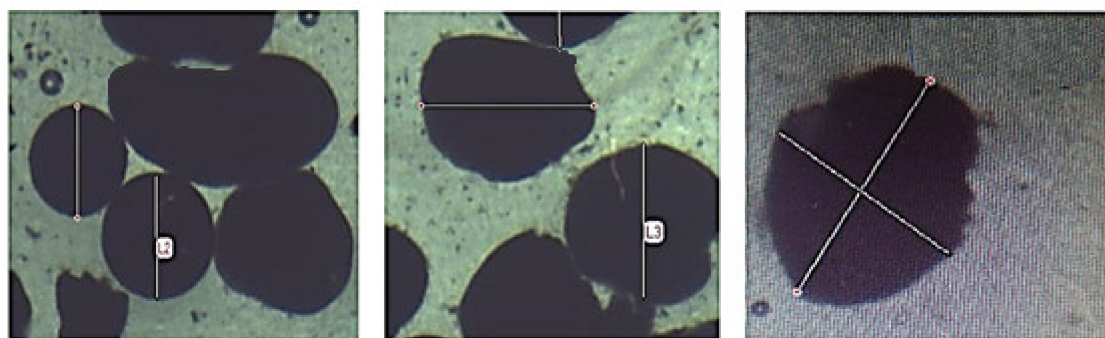
granular sludge. It is hypothesized that there are branches and sub-branches of pore networks inside the granule, which lead to a volcano like opening on the surface of the granule. These openings could be one or more than one and aid in higher mass transfer inside the granule. A larger pore opening will lead to higher intake of substrate by the microbial population [23]. Our results are in agreement with previous studies.



**Figure 6.** Biogas production replicate test.

### 3.4. Single Granule under the Same COD Level

The aim of this test was to investigate the behavior of anaerobic granules under the same COD concentration and validate whether biogas production remains same in the micro reactor. Samples of the measured granules are shown in Figure 7.



**Figure 7.** Selection of granules based on size.

The granules were selected randomly for this test. Based on initial physical examination, granules with similar size range were separated into groups. Afterward, three granules of specific size were chosen for the micro experiment. The size of individual granules was measured as 0.88, 1.32, and 1.75 mm, respectively. As it was a novel method, it was necessary to validate the test and the repeatability of the results. Therefore, under similar conditions, i.e., COD 3000 mg/L, the same sludge was tested twice to assure the stability and reliability of the test. Figure 8 illustrates the bubble volume of a granule under COD 3000 mg/L. The two curves were almost the same, thus, confirming that under the same conditions the granular sludge will produce a similar amount of biogas. The results show that this new method is reliable as evidenced by  $R^2 = 0.998$ .



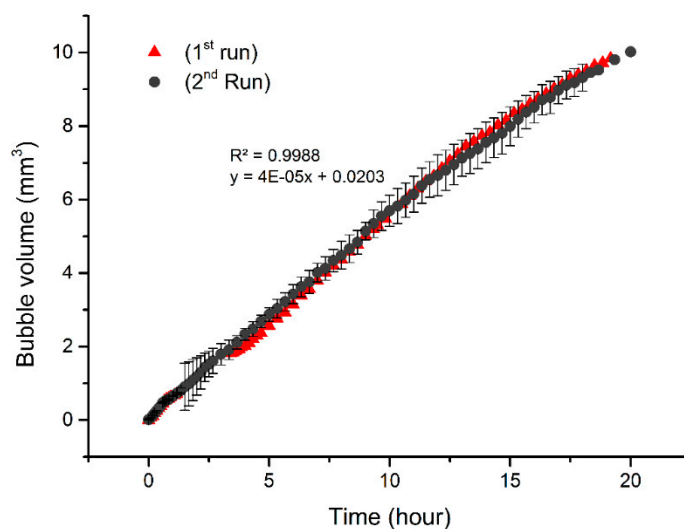


Figure 8. Bubble volume of a single granule in the micro reactor.

### 3.5. Biogas Production of Different Granules under the Same COD Level

The aim of this test was to understand the biogas production of different-sized granules and observe the correlation between size and biogas production. The size of each granule was 1.31, 1.47, and 1.75 mm, respectively. The biogas production of each single granule is shown in Figure 9. At COD 3000 mg/L, a single granule was tested for a time interval of 30 h to see the biogas production. The maximum biogas was recorded for 1.476 mm granules i.e., 0.053 m<sup>3</sup>/kgSS. The biogas production for 1.32 and 1.75 mm granules was 0.023 and 0.012 m<sup>3</sup>/kgSS, respectively, which is significantly low.

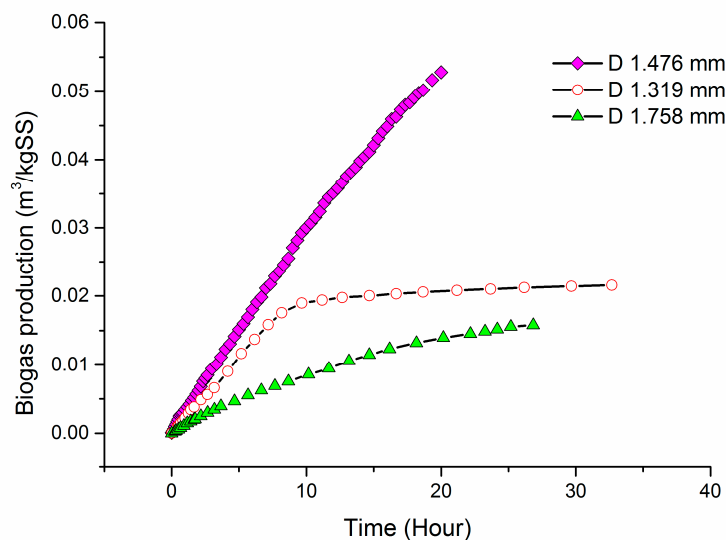


Figure 9. Biogas production of different granules.

It was noteworthy that in the first 10 h, the granules underwent a rapid biogas production process and later became fairly stable. The biogas production for 1.319 and 1.758 mm granules became fairly stable after 20 h. The results indicate that granules have significantly different behavior when exposed to same conditions. This can be attributed to the transformations in the granule microstructure during the formation process [15]. During the granulation process, the biofilm layer formation is dependent on many factors such as hydrodynamic condition, shear forces, and the presence of an active microbial community. During the maturation stage, the original bacterial colonies continue to grow while other dispersed bacteria may also adhere to the embryonic granules [33]. There is no obvious relation

between biogas production and granule size at this stage. This preliminary result laid the foundation to further investigate biogas production and the mass transfer of single granule.

### 3.6. Biogas Production of a Single Granule under Different COD Levels

For further understanding, biogas production behavior of a single granule diameter (1.75 mm) was tested under different COD concentrations. The initial CODs of the substrate added to the micro reactor were 586, 1700, and 6700 mg/L, respectively. At first, initial COD 586 mg/L was tested twice, then the initial COD 1700 mg/L was tested twice, and finally the initial COD 6700 mg/L was tested. A higher COD concentration indicates greater organic load on the anaerobic granule. It is noteworthy that Extra polymeric substances (EPS) content is related to biogas production in an anaerobic granule. A recent study reported high EPS content in smaller granules, thus, nonfunctional bacteria were predominant and hindered the biogas production process [43]. Our results are in agreement with the cited study.

The cumulative curve of biogas production over time is shown in Figure 10. The results of biogas curves were similar to the general rule of biogas production of granular sludge as discussed in the previous section, i.e., accumulative increase in biogas production is gradual and slow. Another noteworthy result is that increase in organic load resulted in higher biogas production in granule. Hegde and Trabold [44] studied the biogas potential of cafeteria waste under variable organic loading rate. The authors reported that the maximum specific methane yield (SMY) was  $363 \text{ mL gVS}^{-1}\text{d}^{-1}$  at an organic loading rate (OLR) of  $2.8 \text{ gVSL}^{-1}\text{d}^{-1}$ , and acid whey, waste energy drinks, and waste bread resulted in a maximum SMY of 455, 453, and  $479 \text{ mL gVS}^{-1}\text{d}^{-1}$ , respectively, and it was possible to achieve stable digestion at OLR as high as  $4.4 \text{ gVSL}^{-1}\text{d}^{-1}$ . The gradual increase of biogas production under higher organic load was in line with our findings.

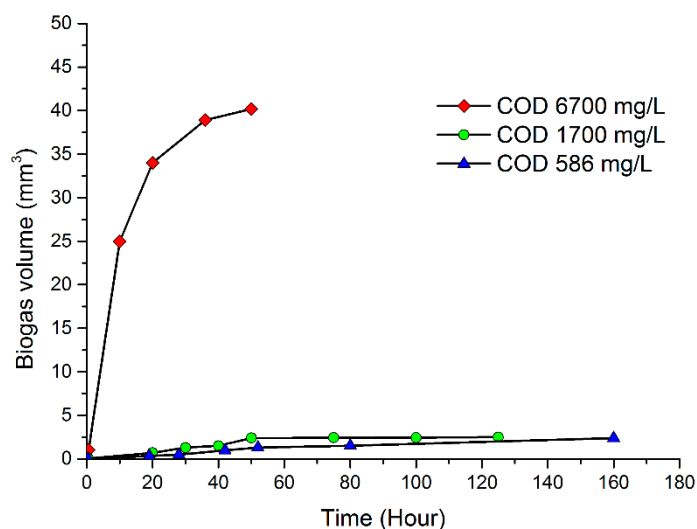


Figure 10. Biogas volume under different COD concentrations.

Figure 11 demonstrates the change in biogas production under different organic loads. The results revealed that the biogas production rates of the initial COD 586, 1700, and 6700 mg/L were 0.0108, 0.0236, and  $0.1007 \text{ m}^3/\text{kg COD}$ , respectively. Interestingly, at micro level the biogas production rate is high when subjected to higher organic loading. By the time of completion of the experiment, biogas production rates for the three groups were 0.036, 0.034, and  $0.101 \text{ m}^3/\text{kg COD}$ . It can be seen that the two groups with low organic loading, i.e., COD of 586 and 1700 mg/L, had close biogas yields. It was noticed that at lower concentrations, the biogas was produced fast and after a certain period the production became stable. This is due to the already hydrolyzed substrate which is consumed by the bacterial community. For maximum organic load of 6700 mg/L, the experiment was discontinued at



47 h, because of slower but higher biogas production. The slow production is due to slower molecular movement inside the granules that require longer time to reach to the core of the granule. However, the active microorganisms (methanogens) on the surface of granule instantly produce biogas when exposed to higher organic loading. Alfa et al. [45] studied the biogas potential for cow manure, poultry, and lemon grass organic wastes and concluded that poultry waste resulted in higher biogas after increasing the organic loading in the reactor.

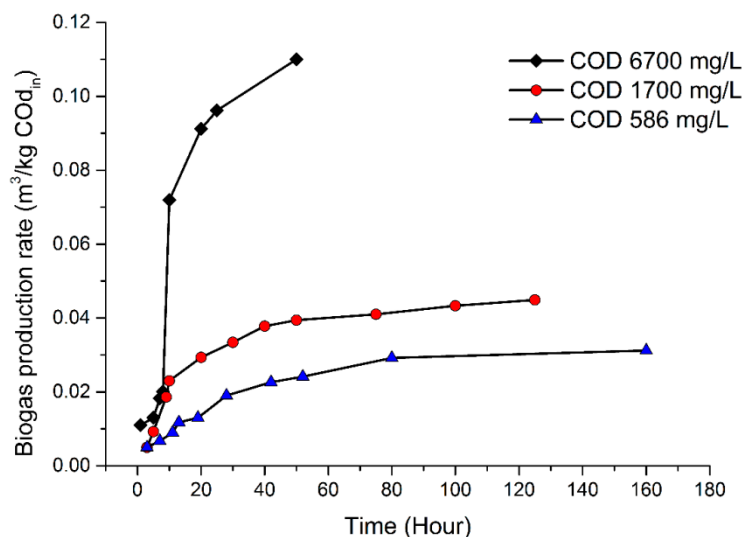


Figure 11. Effects of COD concentration on biogas production.

### 3.7. Mass Transfer Rate

In order to further understand the unique behavior of a single anaerobic granule, mass transfer rates were calculated by applying modified Fick's law for molecular diffusion. The mass transfer rate for a 1.75 mm granule was calculated under COD concentrations of 568, 1700, and 6700 mg/L, respectively. The results were in accordance with the trend of biogas production. It was noticed that mass transfer rates were a function of organic loading. The mass transfer rates were calculated as  $1.82535 \times 10^{-12}$ ,  $5.29538 \times 10^{-12}$ , and  $2.087 \times 10^{-11}$  mg/s whereas mass transfer rates were highest at 6700 mg/L as presented in Figure 12. It was observed that mass transfer increased when the organic loading was increased for different granules.

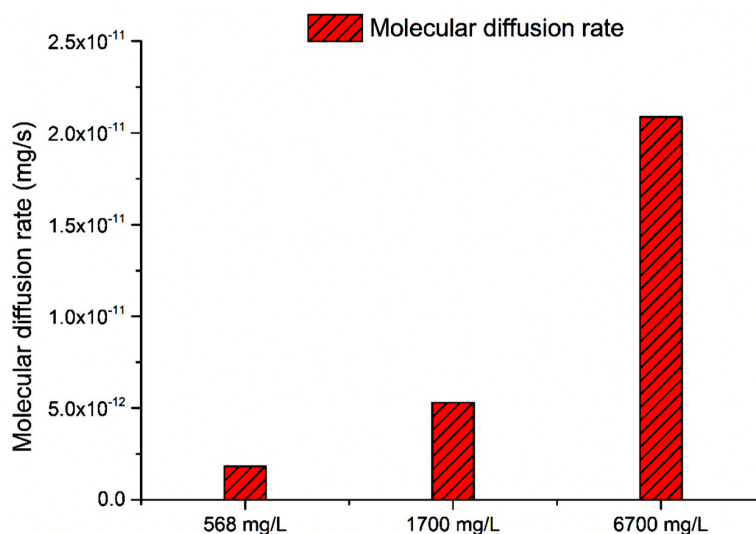


Figure 12. Mass transfer rate of a single granule.

Mass transfer limitation is related to the porosity of sludge granules. Granule size and its distribution is highly important the biogas production and reactor performance due to organic loading distribution inside the reactor [36]. Wu et al. [29] investigated the pore structure of different-sized anaerobic granules and reported that big granules had well-developed pore structure which helps in faster mass transfer process inside the granule. Furthermore, big granules had high density which probably related to the compactness of microbes. This might further enhance the substrate transfer in granules due to the short diffusion distance among those closely bounded microbes. Thus, large granules generate biogas with a high production rate.

As the mass transfer process was purely molecular diffusive, thus at a high organic concentration of 6700 mg/L, the biogas produced was slower, but the overall yield was much higher than that at 586 and 1700 mg/L. It is noteworthy that the magnitude of molecular diffusion is small due to the single granule and the scale of the experiment at micro level. The major mechanism for mass transfer under the static condition is molecular diffusion [21,46]. However, in large reactors the granules are in large volume, thus, the accumulative molecular diffusion is higher. Larger granules had multi-layered internal microstructures with higher acetoclastic methanogenic activities than smaller granules [25].

From the figure it can be inferred that higher organic loading results in a better mass transfer condition. In addition, the larger the granules the higher the mass transfer once acclimatized in the reactor. Current literature reports that large granules have a mature and higher microbial consortium which contributes to higher intake of the biodegradable substrate [33]. The pore-size distribution in an anaerobic granule strongly indicates mass transport limitation for large granules. The pores develop due to gas pressure and substrate limitation causing bacterial decay. Our results are in agreement with similar studies. A molecular approach was used in combination with electron microscopy to characterize the microbial consortia in a laboratory-scale terephthalate-degrading UASB reactor [25]. Jensen et al. [47] predicted the biogas potential in a laboratory-scale experiment and extrapolated it for large reactors and concluded similar results. An increase in organic loading in the reactor lead to higher biogas potential which reflects better mass transfer in the granule [48]. The results of this study results are in agreement to previous studies explaining the fundamental mass transfer mechanism.

#### 4. Conclusions

The biogas production and mass transfer phenomena were studied for individual anaerobic granules in a micro reactor. The morphology of granules was observed under a microscope and well-grown granules were selected for biogas study. Furthermore, real-time microscopic study explained the biogas bubble production and detachment process for the first time. The granule size ranged between 1.32, 1.47, and 1.75 mm and it was observed that different-sized granules had different mass transfer rates but granules equal to or larger than 1.75 mm were considered mature and recommended for large reactors. The biogas production rates of the initial COD 586, 1700, and 6700 mg/L were 0.0108, 0.0236, and 0.1007 m<sup>3</sup>/kg COD, respectively. At the highest organic load of 6700 mg/L, the mass transfer rates were calculated as 1.82535E<sup>-12</sup>, 5.29538E<sup>-12</sup>, and 2.087E<sup>-11</sup> mg/s. The low magnitude of mass transfer is attributed to the absence of external hydrodynamic forces. Therefore, mass transfer is purely molecular in nature in the micro reactor. The higher mass transfer is attributed to a bigger microbial consortium as reported in earlier studies. It was interesting to observe that size of the single granules was similar to each other, but biogas production rate was different for anaerobic granules under same COD conditions, but increasing COD concentration improved the mass transfer rates in the granule which enhanced the volume of biogas in the micro reactor. Thus, it is concluded that large granules and higher organic loading lead to better mass transfer and biogas production in the reactor. These results will enhance the understanding for better design and performance of anaerobic reactors for biogas production.

**Author Contributions:** This work is primarily based on the Ph.D. research work of Z.A. at Tsinghua University, Beijing. This Ph.D. work was supervised by W.J. H.Y. curated and provided experimental support and A.B.

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