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Enhanced Onsite Treatment of Domestic Wastewater Using an Integrated Settler-Based Biofilm Reactor with Efficient Biogas Generation

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Abstract: The growing population and increasing urbanization have led to a surge in domestic wastewater generation, posing significant challenges for effective and sustainable treatment. The present study demonstrates a novel and sustainable approach for the onsite treatment of domestic wastewater using an integrated settler-based biofilm reactor (ISBR) with efficient biogas generation. The ISBR provides an optimized environment for the growth of biofilm, facilitating the removal of organic pollutants and pathogens. Moreover, the ISBR enables the recovery of a valuable resource in the form of biogas, thus enhancing the overall utility of the treatment process. The performance of the ISBR was comprehensively evaluated at laboratory scale through treating the actual domestic wastewater generated from the hostel of Manipal University Jaipur. The ISBR system was operated under an ambient environment at a hydraulic retention time (HRT) of 24 h. The results demonstrated remarkable efficiency in terms of chemical oxygen demand (COD), total suspended solids (TSS), and coliforms removal, with average removal efficiency being more than 90%. According to the COD mass balance analysis, 48.2% of the influent COD was recovered as bioenergy. The chromatogram revealed a high percentage of methane gas in the collected biogas sample. The field emission scanning electron microscope (FESEM) analysis of the accumulated sludge in the ISBR system depicted the morphology of methanogenic bacteria. Both the experimental and theoretical results confirmed the feasibility and sustainability of the ISBR system at the onsite level.

Keywords: anaerobic treatment; bioenergy; domestic wastewater; gas chromatography; mass balance

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1. Introduction

Adequate sanitation facilities are a basic human right and a crucial component of sustainable development [1]. However, about 8% (616 million) of the global population still does not have access to improved sanitation. Improved sanitation includes 54% safely managed facilities, 24% basic facilities, and 7% limited facilities. Basic and limited facilities (septic tanks, pit latrines, flush latrines, etc.) have low pollutant removal efficiency, leading to the degradation of the surrounding environment and water bodies. Therefore, there is a need for innovative and sustainable approaches that can be applied in a variety of situations [2]. Onsite treatment systems have evolved as a viable and practical alternative, providing domestic wastewater management solutions tailored to specific local requirements [3].

Onsite treatment means the management of domestic wastewater close to its source of generation, aimed at alleviating the challenges of establishing and maintaining effective

sewage infrastructure, particularly in remote or economically disadvantaged areas. Septic tanks, conventional septic tanks, composting toilets, pit latrines, biofilters, constructed wetland-based systems, and other technologies and techniques are included in these systems. As far as the popularity of various onsite treatment technologies is concerned, approximately 40% of the world's population still depends on these systems. But due to some serious drawbacks of these antiquated onsite sanitation practices, they fail to fulfil disposal standards [2]. The main contributing factors of design flaws and poor maintenance are the main concerns that lead to the low pollutant removal efficiency of these onsite sanitation systems. Due to low treatment efficiency, pollutants significantly contaminate the surrounding water bodies [4]. The lack of access to proper sanitation systems continues to be a global issue and affects about 4.5 billion people (WHO and UNICEF, 2017). Inadequate sanitation endangers public health, with diarrheal infections being the most serious concern [5].

Onsite treatment systems like septic tanks and latrines are frequently utilised in low- and middle-income countries (LMICs), where more than 38% of the world's population lives [5,6]. Unfortunately, the prevalence of poor sanitation in these places is an important factor in the casualties and disabilities caused by diarrhoea. According to Pruß-Ustün et al. (2019), poor sanitation exclusively resulted in 25.8 million disability-adjusted life years (DALYs) and 431,720 diarrhoea-related deaths in LMICs in 2016 [7,8]. To tackle these challenges and improve onsite sanitation conditions, there is a need to work out the functionality, limitations, benefits, and potential for widespread adoption of onsite sanitation systems. Even today, anaerobic treatment systems remain a viable and satisfying solution for onsite treatment. Additionally, other benefits of anaerobic treatment and digestion are a smaller electricity requirement, less sludge formation, affordable and effective treatment with high pollutant removal efficiency, and the large-scale production of biogas [9,10]. In the 21st century, where shortages of fresh and potable water are very common, it is required to recycle treated wastewater for household purposes. Therefore, it is necessary to improve treatment practices through developing efficient and sustainable systems for domestic wastewater (DWW) treatment [11]. In developing countries like India, where 68% of the population lives in villages (UBA-2019) [12,13], people are still dependent on conventional systems like septic tanks or pit latrines for the treatment of domestic wastewater [14,15]. But the removal efficiency of these conventional methods is very low (UNEP-2004). Although the Indian Government has constructed more than 10 crores of twin pit latrines under the Swachh Bharat Mission after 2014 with a mission of making India open defecation free, their treatment efficiency and maintenance is still a problem [16,17]. There is no doubt that at the centralized level, there are a lot of technologies available to handle and manage wastewater. It is also true that the anaerobic digestion (AD) process has been widely applied in the treatment of wastewater and biomass to provide more sustainable and cost-effective treatment at the onsite level. It also leads to higher bioenergy recovery [18,19]. Temperature also plays a major role in the AD process for biogas generation from sludge, and external energy is required to heat the digester in low-temperature areas [20–23]. Anaerobically digested organic matter exhibits the highest methanogenic activity under either mesophilic (30–40 °C) or thermophilic (50–60 °C) environments [24–26]. At low temperatures, microbial growth is highly reduced, which increases the need for longer hydraulic retention time (HRT) and sludge retention time (SRT) [21]. The concentration of organic matter (low and high) at ambient temperatures can be removed to a great extent using modern technologies, such as the bio-toilet, anaerobic fixed film reactors, and anaerobic sludge blanket reactors (UASBs) [27], which produce water and biogas from the COD content of wastewater even at low ambient temperatures. Therefore, prior to implementation of these types of technology, proper research should be carried out to make them more suitable and sustainable.

In recent years, there has been an increasing interest in applying anaerobic membrane bio-reactor (MBR) technology for the treatment of low-strength municipal sewage at low temperatures [28–31]. However, most of these studies are based on lab-scale experimental

setups with feeds of synthetic wastewater [32,33], where the reactor's operating volume is low and the investigations are limited to simple indicators. Although some studies have reported operation at temperatures below 20 °C [28,34,35], not too many of them have been performed on the generation of biogas at low temperatures with actual sewage treatment at the onsite level.

The generation of renewable energy from domestic wastewater is gaining popularity as a viable option for both energy generation and wastewater treatment. To derive energy from wastewater, many technologies have been investigated, including anaerobic digestion, microbial fuel cells, and anaerobic membrane bioreactors [36,37]. These techniques not only help to reduce environmental impacts and greenhouse gas emissions, but they also provide the potential for resource recovery, such as biogas production and fertilizer recycling. The combination of renewable energy production with wastewater treatment has enormous potential for meeting global energy demands while creating a circular economy and reducing the environmental impact of domestic wastewater disposal [38].

The anaerobic digestion of wastewater to produce biogas is one promising option where wastewater contains a high amount of COD content and microbes produce biogas, which is mainly composed of methane (CH_4) and carbon dioxide (CO_2). Another option is COD mass-balancing. It is an essential method to quantify the distribution and transformation of chemical oxygen demand (COD) inside a waste treatment system. The various kinds of COD and their individual contributions to the overall system can be quantified using mass balance methods, which can provide important insights into COD contaminants and the efficacy of energy during the treatment process [39].

The present study focused on the development of an efficient integrated settler-based biofilm reactor (ISBR) as a sustainable solution of wastewater management at the onsite level. The ISBR system combines two key processes, anaerobic digestion and biofilm formation, that help in the significant removal of pollutants. It is designed to treat domestic wastewater and provide an efficient solution for water sanitation challenges. In order to check the performance in terms of pollutant removal efficiency, feasibility, operational viability, and the suitability of the designed system, it was operated in actual ambient conditions.

The performance of the lab-scale ISBR was examined for 120 consecutive days under steady flow conditions at a hydraulic retraction time of 24 h. The objectives of the present study were (1) to investigate the long-term performance of ISBR fed in an ambient environment, (2) comprehensively analyse methane content through COD mass balance during the operation of the ISBR, (3) measure the percentage of methane found in biogas from the experimental setup, and (4) examine the morphology of existing bacteria within the accumulated sludge using FESEM analysis. The scope of this onsite system is to provide a sustainable solution for water sanitation challenges in developing or low-income countries. The study aims to provide valuable information about the ISBR as an advanced onsite wastewater treatment system with a strong emphasis on sustainable biogas production and efficient pollutant removal.

2. Materials and Methods

2.1. Experimental Setup

A laboratory-scale ISBR was fabricated using transparent acrylic sheets in a rectangular shape. The overall dimensions of the reactor were 564 mm long, 280 mm wide, and 450 mm high with a total volume of 72 L and working volume of 57 L. This reactor was divided into three chambers, which were connected in a series with the help of acrylic pipes. The typical connection of the pipes makes the flow in an upward direction in all the chambers, as has been clearly shown in Figure 1.

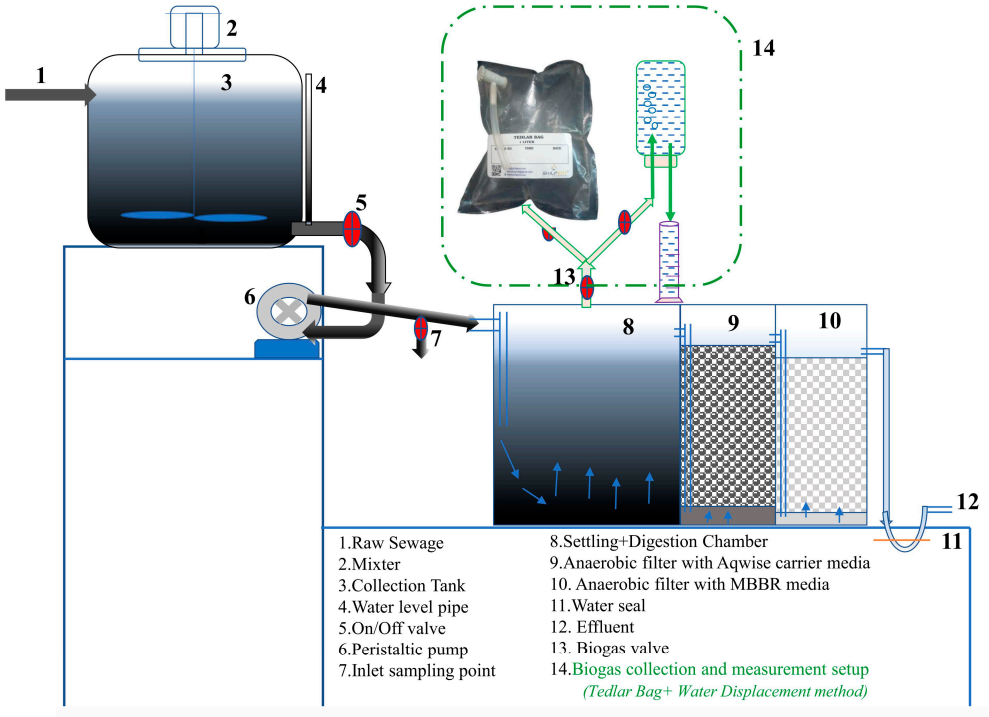

Figure 1. Schematic diagram of laboratory-scale ISBR.

Chamber 1 works as a settler and digestion chamber and has a biogas collection knob at its centre. The 2nd and 3rd chambers have two different types of filter media. In the second chamber, Aqwise carrier media was used, having a surface area of 650 m^2/m^3. In the 3rd chamber, MBBR media was used, which had a surface area of 250 m^2/m^3 [40]. These plastic media provide a large surface area for the biofilm to grow on and are thus sometimes also called biofilm filters. The biogas knob is directly connected to a Tedlar bag for collection of biogas. The water displacement method was used for the quantitative measurement of methane.

2.1.1. Process of Reactor Operation

This onsite sanitation system (ISBR) worked on actual sewage, which was collected from the collection tank of 1 MLD sewage treatment plant at a Manipal University Jaipur hostel with the help of a sewage submersible pump. Using this pump, we fed 80 L of sewage daily into the collection tank of the lab-scale unit. Then, 40 mL/min sewage was fed to the reactor continuously (24 × 7) with the help of a peristaltic pump. This study worked on 24 h of hydraulic retention time (HRT) at a steady flow condition after the start-up phase. The reactor took 50 days to stabilize after being seeded with bio-gas digester sludge taken from the biogas digester at Manipal University Jaipur.

During the operation of the ISBR lab-scale setup, we initially collected sewage from Manipal University Jaipur's sewage treatment plant and collected it into the collection tank of the lab-scale setup, as shown in Figure 1. After that, we used a peristaltic pump to feed the sewage into the ISBR's first chamber from the collection tank, which was designed as an anaerobic settling chamber. The functioning of the ISBR settling chamber involves several fundamental mechanisms. When sewage enters the settling chamber, the lack of oxygen encourages the growth of anaerobic microorganisms. Through fermentation and acid genesis, these microorganisms convert complex organic substances into simpler forms. As a result, biogas is produced, which is mostly contains methane and carbon dioxide [41]. At the same time, the solid particles in the wastewater, such as suspended solids, settle due to gravity [42]. Because of the slower flow rate and upward movement of sewage in the chamber, heavier solids separate and settle down to the bottom. Partition walls and

an upward flow design can be utilised to improve the sedimentation process and enhance interaction between the settled solids and the sewage.

Thereafter, sewage enters the second chamber of the ISBR through a pipe fitted in the top-to-bottom direction from the first chamber but exits in the upward direction. The third chamber follows the same design pattern. The second and third chambers are filled with two different types of plastic media, as mentioned in the Section 2.1.

In ISBR systems, these plastic media are used as biofilm filter materials. They are made up of especially HDPE plastic materials with a large surface area for the growth of biofilm, which is a population of microorganisms that attach to the surface of the media. The shape and chemistry of the plastic media promote the formation of a dense and diverse biofilm, allowing for efficient wastewater treatment. The detailed investigation of reactor stabilization and biofilm is published in my earlier publication [13,14].

As wastewater passes through the biofilm filter, the microorganisms present in the biofilm metabolise organic particles and pollutants, breaking them down into simpler compounds. This biological process facilitates the removal of pollutants from the wastewater and the improvement of its quality. The structure of the plastic media encourages good contact between the wastewater and the biofilm, allowing for effective microbial activity.

The durability, lifespan, and resistance to fouling are all advantages of employing plastic media as a biofilm filter. To maximise surface area and provide adequate space for the biofilm to develop, the plastic material is frequently created with precise geometric designs, such as rings, tubes, or cross-shaped pieces. Because of this increased surface area and the activity of the biofilm, plastic media are an effective and extensively utilised alternative in biofilm reactors, which increases the performance of the reactor [43].

Finally, the third chamber was connected to a U-shaped effluent pipe, which acted as the pathway for the treated wastewater. This U-shaped pipe was designed with a water seal to prevent biogas from escaping with the effluent. Due to this, the biogas produced within the system was easily collected. To collect the biogas generated in the reactor, the gas was collected into a Tedlar bag via a biogas knob. The Tadlar bag is a temporary storage solution for small-scale biogas production.

To evaluate the reactor's performance, we collected raw sewage directly from the reactor's entrance and effluent samples from the outlet. Following that, the samples were taken to the Environmental Engineering Laboratory for detailed testing and analysis. This approach allowed us to assess the reactor's efficacy in treating raw sewage and provided vital insights into its functioning.

2.1.2. Analytical Procedure and Instrument

The analytical parameters such as pH, temperature, alkalinity, volatile fatty acids (VFA), total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and volatile suspended solids (VSS) were measured according to the standard methods of examination for water and wastewater [44], except VFA [45], which was measured twice a week. The pH and temperature were analysed using an HQ 40d multi-parameter instrument (Hach-USA, Loveland, CO, USA). For BOD (3 days incubation at 27 °C), we used the BOD incubator (Tanco-PLT141, P.L. Tandon & Co, New Delhi, India). The COD was digested in the COD Digester (DRB-200, Hach, Loveland, CO, USA) and was analysed for results using a UV-VIS Spectrophotometer without RFID (Model no. DR6000 Hach-USA). For microbial analysis, we used specific types of broth and agar for total coliform (enumerated by the multiple tube fermentation technique) [44] and faecal coliform (M-FC Broth Base, HIMEDIA-M1111, HiMedia Laboratories, Maharashtra, India). The pathogenic bacteria, such as faecal bacteria, E-coli, total coliform, and faecal coliform, were extracted via the membrane filtration method and E-coli with EMB agar base (HIMEDIA m 301, HiMedia Laboratories, Maharashtra, India). All these instruments and analytical facilities were available at the Environmental Engineering lab of Manipal University Jaipur.

The generated biogas was measured via the water displacement method. Methane and carbon dioxide were the other two significant constituents of the biogas. The biogas

subsequently went through 1.5% NaOH solution for eliminating CO_2, and the measure (in mL) of the displaced NaOH was recorded as methane [46].

2.1.3. Gas Chromatography (GC) for Biogas

GC analysis was performed to determine the methane available in biogas. During the analysis, we used a GC-2014 (Shimadzu-01623, Shimadzu, Kyoto, Japan), which was equipped with TCD technique, a gas sampler (MGS-5, Shimadzu, Kyoto, Japan), and porapak-n column (length—2 m and inner diameter—2 mm). The instrument is available at the Central Analytical Facility, Manipal University Jaipur. The gas sampler (MGS-5, Shimadzu, Kyoto, Japan) discharges 1 mm of biogas, which is connected to the GC. The programmed method was used for analysis where the injection temperature was 100 °C, column temperature 40 °C, TCD temperature 150 °C, current 70 mA and a running time of 5 min. Nitrogen gas was used as the carrier gas at a flow rate of 25 mL/min. The concentration of methane in biogas was detected on behalf of standard methane gas (99.9999% pure), which was purchased from Ankur Specialty Gases and Technologies, Jaipur, India.

2.1.4. Preparation of Sludge for FESEM

The morphology of the extracted sludge was investigated using a Field Emission Scanning Electron Microscope (FESEM, Photometrics, Inc., Huntington Beach, CA, USA). The sample was first fixed in phosphate-buffered solution with 2.5% (*w*/*v*) glutaraldehyde at 4 °C for 1 h. This was then sterilized through a graded series of acetone and water mixtures (10%, 25%, 50%, 75%, 90% and 100%). These samples were brought to equilibrium in 10 min and were dried up to the critical point. Before each FESEM analysis, the samples were coated with aurum [39,47]. Finally, the samples were tested using FESEM (NOVA NANO SEM-450, Photometrics, Inc., Huntington Beach, CA, USA).

3. Results and Discussions

3.1. Overall Performance of ISBR

The ISBR system was started via inoculating the biogas digester sludge with COD and VSS concentrations of 9.6 and 7.13 g per litre, respectively. From 1 to 50 days, the system's removal efficiency in terms of COD was around 30 to 70%. It was the initial phase of the system in which microbes and biofilm grew on filter media. Later, the system attained a stable condition. Table 1 shows the average results of the physical, chemical, and biological parameters of the system after the initial phase [13].

Table 1. Treatment performance with average concentration of physiochemical and microbial parameters.

Parameters	Influent	Effluent	Removal Efficiency (%)
pH	7.42 ± 0.43	7.51 ± 0.31	-
Temperature(°C)	30 ± 50	30 ± 56	-
Alkalinity(mg/L as $CaCO_3$)	372 ± 38	380 ± 25	-
VFA (mg/L)	16 ± 4	12 ± 3	-
TSS (mg/L)	391.30 ± 67.09	24.36 ± 2.72	93.47 ± 1.76
BOD (mg/L)	336.16 ± 40.13	28.93 ± 3.41	91.30 ± 1.27
COD (mg/L)	639.39 ± 98.83	57.40 ± 4.08	90.78 ± 1.68
VSS (mg/L)	285.71 ± 46.9	-	-
TC (MPN/100 mL)	$5.1 \times 10^8 \pm 1.2 \times 10^8$	$4.2 \times 10^7 \pm 1.2 \times 10^7$	91.76 ± 1.5
FC (CFU/100 mL)	$8.1 \times 10^7 \pm 2.1 \times 10^7$	$6.6 \times 10^6 \pm 1.2 \times 10^6$	91.85 ± 1.6
E-coli (CFU/100 mL)	$8.5 \times 10^6 \pm 1.0 \times 10^6$	$1.4 \times 10^5 \pm 1.9 \times 10^5$	83.52 ± 3.1

Average ± standard deviation

This system worked on actual sewage, which was generated at the hostel of Manipal University Jaipur. The pH value of raw sewage was around 7.42 ± 0.43, and in the effluent, it slightly increased to 7.51 ± 0.31. The formation of biogas and fatty acids led to an increase in the value of pH [36]. The CO_2 present in the biogas reacted with water and increased the carbonate alkalinity due to which total alkalinity also increased from 372 ± 38 to 380 ± 25 mg/L as $CaCO_3$. The value of the pH decreased in the first chamber of the system. The VFA results showed that hydrolysis, acidogenesis, and acetogenesis occurring in the reactor were due to these biochemical activities [48,49]. In the final step, these acids were converted into biogas through the methanogenesis process with the help of methanogenesis anaerobes [50], which is shown in the last FESEM images of the accumulated sludge.

The average feed concentrations of raw sewage in terms of TSS, BOD, and COD were 391.30 ± 67.09, 336.16 ± 40.13, and 639.39 ± 98.83 mg/L, respectively. After treatment in the ISBR, the concentration of TSS, BOD, and COD in the effluent were found to be 24.36 ± 2.72, 28.93 ± 3.41 and 57.40, ± 4.08 mg/L, respectively, which fulfils the disposal norms of the National Green Tribunal (O) (NGT-2019) India. During the analysis, we observed significant variations in the inlet concentration, which was due to the changes in the hostel diet menu. The system was subjected to organic shock loading, which initially caused a lot of variation in the inlet effluent concentration, but later, the system attained stability. The results are shown in Figure 2.

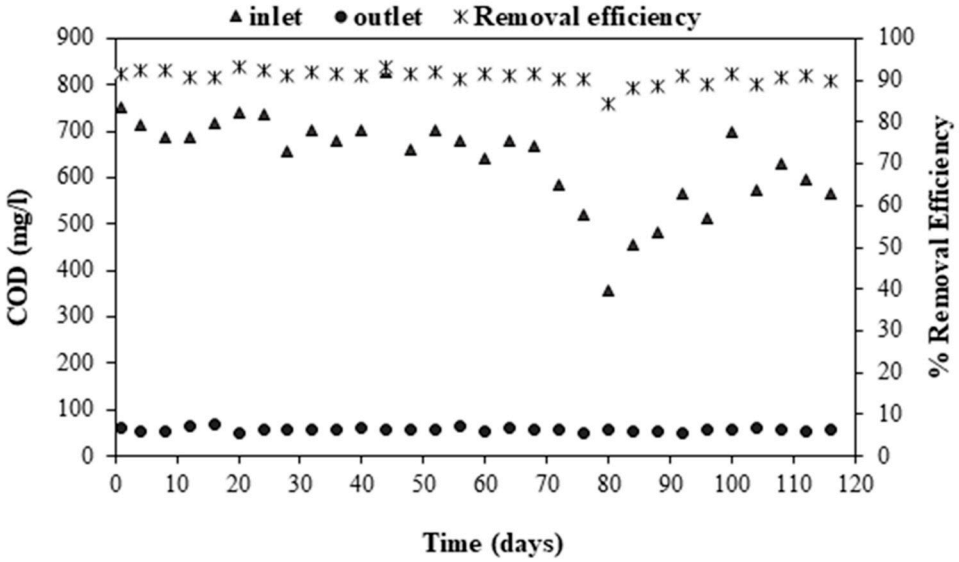

Figure 2. Evaluation of COD concentration of influent and effluent with removal efficiency.

During the study, it was found that the biodegradation of organic materials and biogas generation rate were influenced by temperature, which varied from 19 °C to 35 °C. It resulted in 48.2% of total COD being utilized as methane gas. Figure 3 shows the relationship between the COD removal efficiency and the organic loading rate of the system. The overall COD removal efficiency was found to be more than 90%. At the highest organic loading rate of 0.83 kg COD/$m^{3,}$ the removal efficiency was 93.10%, and at the lowest organic loading rate of 0.36 kg COD/$m^{3,}$ the removal efficiency was 84.31%. The linear trend line in Figure 3 shows that as the organic loading increases, the COD removal efficiency improves.

The microbial concentrations of the ISBR are also mentioned in Table 1. Faecal coliforms and E. coli are usually classified as pathogens. During the analysis, their concentrations in the inlet, in terms of total coliform, faecal coliform, and *E. coli*, were $5.1 \times 10^8 \pm 1.2 \times 10^8$, $8.1 \times 10^7 \pm 2.1 \times 10^7$, and $8.5 \times 10^6 \pm 1.0 \times 10^6$, respectively. In the outlet, these were $4.2 \times 10^7 \pm 1.2 \times 10^7$, $6.6 \times 10^6 \pm 1.2 \times 10^6$, and $1.4 \times 10^5 \pm 1.9 \times 10^5$, respectively.

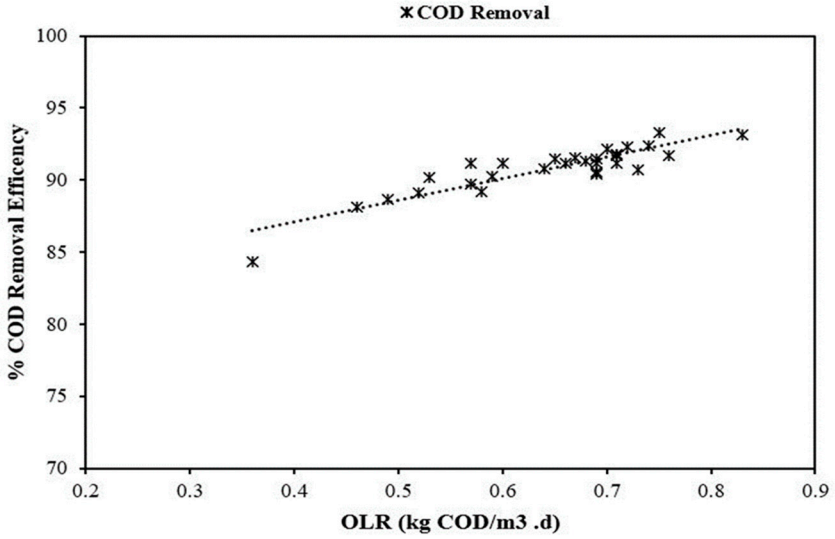

Figure 3. Evolution of COD removal efficiency with variable organic loading rate.

3.2. COD Mass Balance

COD mass balance in wastewater treatment is a systematic method for assessing and quantifying the inflow and outflow of organic matter in a treatment system. It entails determining the concentration of COD in the influent (raw wastewater) and effluent (treated wastewater) at various stages of the treatment process. It also takes into account any variations in COD concentration inside the treatment system as a result of biodegradation, chemical reactions, or other activities.

The COD mass balance is a mathematical expression used to account for the distribution and transformation of COD within a system. It assures that the sum of COD inputs equals to the sum of COD outputs when various forms and components of COD are taken into account. According to the mass balance equation, the total COD entering the system (COD_{inlet}) must be equal to the sum of the COD associated with methane in the aqueous phase ($CODCH_{4aqueous}$), COD associated with methane in the gas phase (COD_{CH4gas}), COD associated with the biomass ($COD_{biomass}$), COD measured in the effluent (COD_{outlet}), and any undetermined COD ($COD_{undetermined}$) that is present [39].

According to the first principle of thermodynamics, neither energy nor mass can dissipate within a system, but the energy and mass balance of systems with defined boundaries must be unchanged [51]. At steady flow condition, mass balance work was carried out at 24 HRT of the system for anaerobic digestion of organic matter present in all three chambers of the ISBR.

In ISBR, COD mass balance was carried out according to Equation (1).

$$COD_{inlet} = COD_{CH4aqueous} + COD_{CH4gas} + COD_{biomass} + COD_{outlet} + COD_{undetermined} \quad (1)$$

$$COD_{inlet} = S_{in} \times Q \quad (2)$$

where S_{in} = mean inlet COD concentration (g/L) and Q is the flow rate (L/d).

The mass of outlet COD was calculated using Equation (3):

$$COD_{Outlet} = S_{out} \times Q \quad (3)$$

where S_{out} = mean outlet COD concentration (g/L) and Q is the flow rate (L/d).

$$COD\text{-}CH_4 \text{ lost(aqueous)} = 4 \times C_{equil} \times \text{flow} \quad (4)$$

where C_{equil} is the concentration of gas dissolved in the liquid at equilibrium (mg/L).

$$C_{equil} = K_H \times Pgas$$

where K_H is Henry's law constant.

$$K_H = 0.384 \times t + 36.44$$

where t is the temperature and Pgas is the partial pressure of the gas above the liquid (0.7–0.8).

Biomass COD was calculated using Equation (5), as suggested by [52].

$$\text{Biomass COD (g/d)} = 0.0568 \times \text{\% COD removal} \times \text{flow (Q)} \quad (5)$$

where Q is feed flow to the reactor at each HRT in m^3/d. Methane COD was determined using Equation (6), as suggested by [52].

$$\text{Methane COD (g/d)} = 780 \times M \times V_{CH4} / (273 + t) \quad (6)$$

where M is % of methane content in the biogas, V_{CH4} is the volume of the biogas produced in litres (L), and t is the temperature in °C.

Mass balance was performed using Equations (1)–(6). On the basis of the mass balance equations, all forms of COD were calculated, which is shown in Figure 4. Mass balance yielded following distribution at 24 h HRT: CH_4 COD (gas phase, 48.18%) > COD undetermined (21.9%) > CH_4 COD (aqueous phase, 20.36%) > outlet COD (3.3%) > biomass COD (0.8%).

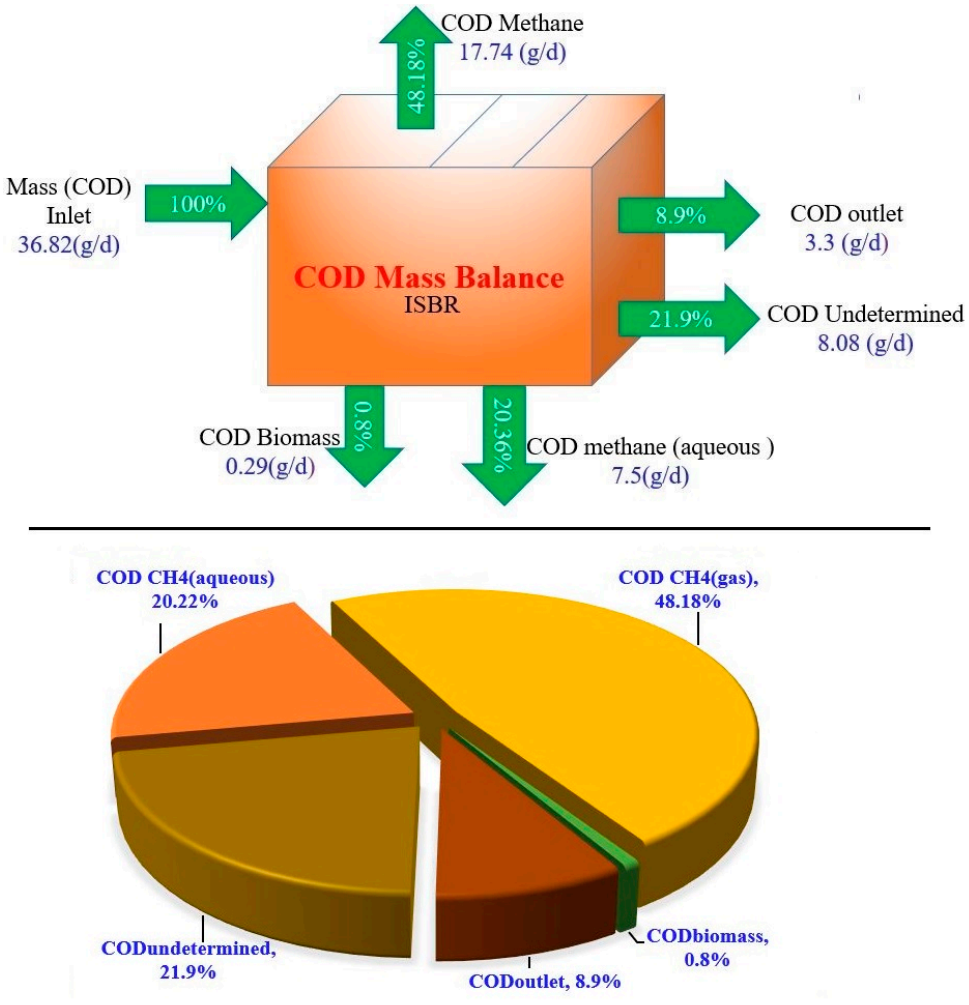

Figure 4. Mass balance and percentage distribution of influent COD in ISBR.

In this study, the practical quantification of biogas production from the ISBR was determined using the water displacement method [53]. The daily volume of water displaced by biogas was measured to be 142 ± 32 mL/day on an average. It is crucial to note that this value varied due to changes in inflow concentrations and temperature effects [54], both of which impacted the rate of biogas generation. These variations indicate the system's dynamic character and the importance of careful monitoring and management of operating parameters to optimize biogas output.

Biogas produced by the system was collected directly in a Tedlar bag, and gas chromatography was carried out to find out the concentration of methane present in it. Figure 5a shows a chromatogram of standard methane gas (99.9999% pure) using single point calibration where the single peak was found at 0.998. But in the case of the sample chromatogram, as shown in Figure 5b, three peaks (at 0.851, 1.077, and 3.030) were detected. Gas chromatography detected 48% of the methane present in the biogas sample with reference to the standard chromatogram.

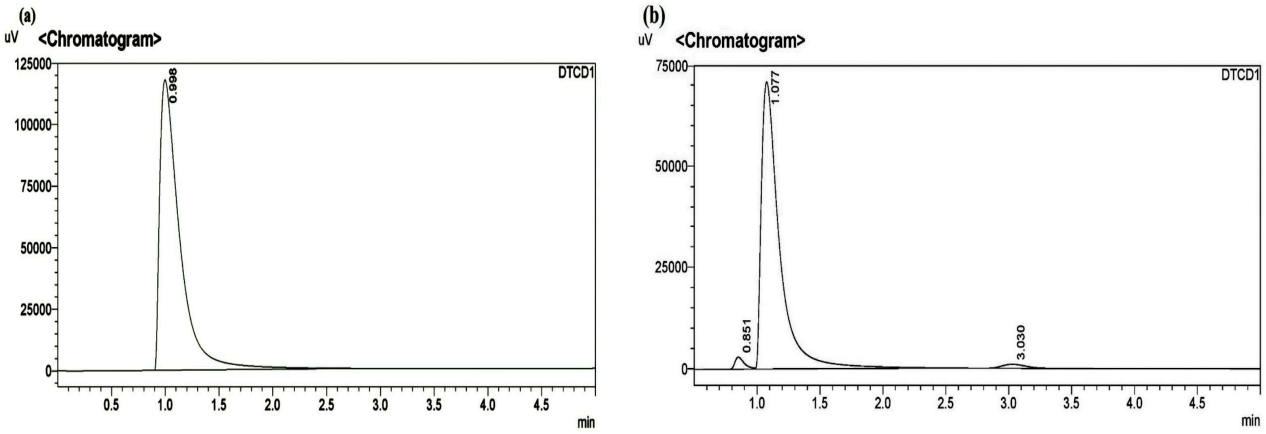

Figure 5. Chromatogram of biogas: (**a**) standard CH_4 and (**b**) gas sample collected from ISBR.

3.3. Sludge Morphology of ISBR

Sludge is a by-product of wastewater, which is formed through the digestion of the organic matter present in sewage. In this anaerobic reactor, the digestion process was carried out by different types of anaerobes, which were detected through FESEM analysis of the sludge, as shown in Figure 6. These microbes play a very important role in digesting the organic matter and producing biogas [13,40].

In the morphology of the first chamber (settler) sludge, ring-shaped and spherical roller-shaped anaerobes were found, and in the second chamber, filamentous cable-type microbes were found. In the third chamber, rod- and ball-shaped anaerobes were found.

The shapes of anaerobic microorganisms are also coccoid shaped, rod–filamentous type, and small balls like methanogens. And it also produces biogas from the reactor. This confirms that, this microorganism is a type of methanogen. In addition, some species of methanogens are able to form biofilms through sticking on media surfaces and making the reactor infective. These methanogens are unique in their ability to survive and thrive in extreme environments with varying pH (6.5 to 8) and temperature (25 °C to 40 °C) conditions. They possess specific enzymes that allow them to carry out the biochemical reactions required for methanogenesis, the biological process that produces methane [55].

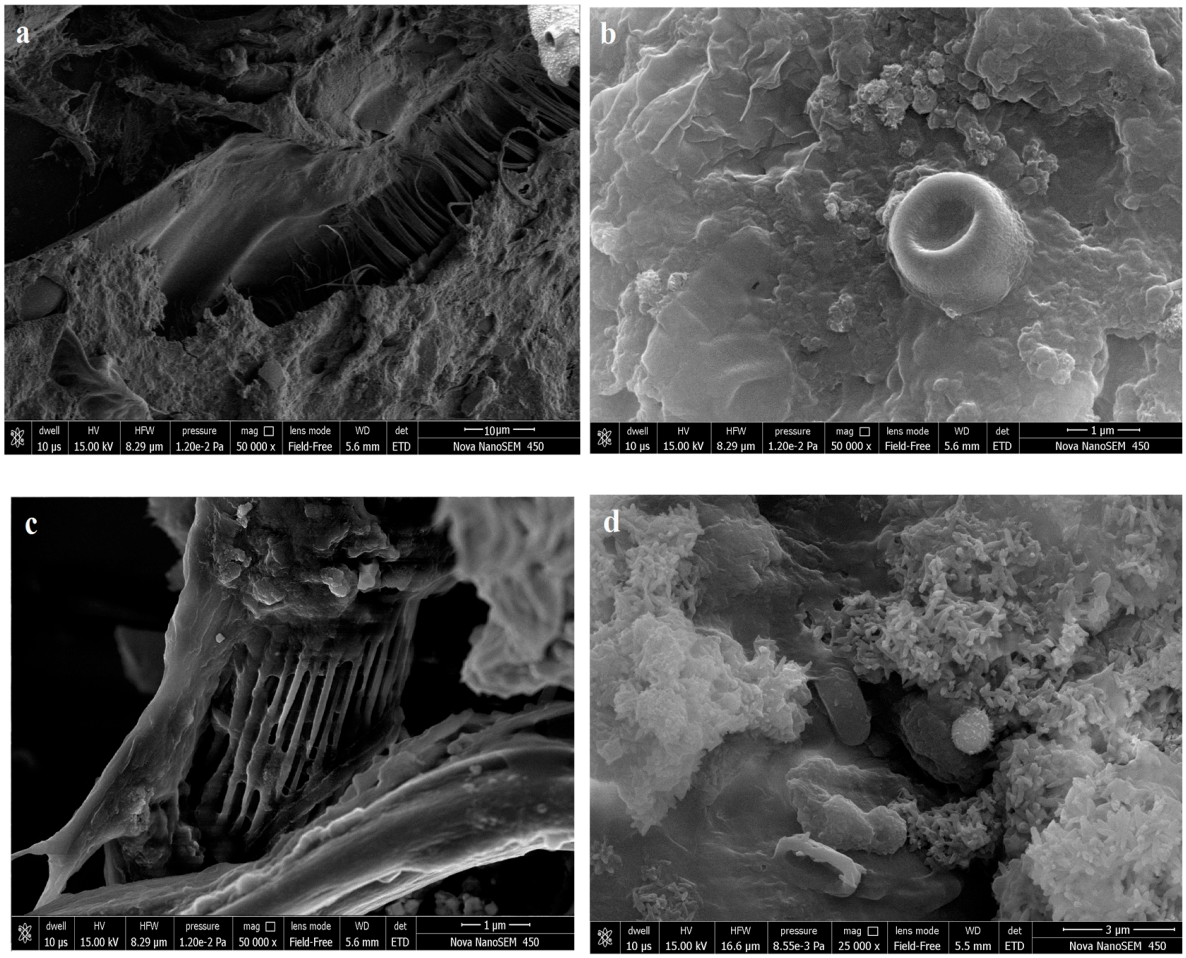

Figure 6. FESEM images of accumulated sludge in ISBR: (**a**,**b**) first chamber morphology—ring-shaped structure, (**c**) second chamber morphology—filamentous cable types of microbes, and (**d**) third chamber morphology—rod and ball shaped microbes.

4. Conclusions

The lab-scale ISBR demonstrated outstanding performance in the treatment of domestic wastewater under steady flow conditions with an HRT of 24 h.

The system achieved high removal efficiencies for TSS, BOD, and COD, with values of 93.47 ± 1.76%, 91.30 ± 1.27%, and 90.78 ± 1.68%, respectively. The biogas yield reached 17.74 g/d of COD removed, indicating efficient energy recovery.

Impressively, approximately 48.18% of the influent COD (639.39 ± 98.83 mg L^{-1}) was converted into CH_4, with only 0.8% converted into COD biomass. This signifies the high potential for generating biogas from the onsite wastewater treatment process. The bacterial removal efficiency was also noteworthy, with percentages of 91.76 ± 1.5%, 91.85 ± 1.6%, and 83.52 ± 3.1% achieved for total coliforms, faecal coliforms, and E. coli, respectively.

The sludge FESEM analysis revealed a wide variety of bacterial morphologies, indicating the presence of a functioning anaerobic treatment system. These findings illustrate the ISBR's effectiveness and practicality for onsite domestic wastewater treatment. The system has high removal efficiency, significant biogas production, and bacterial control, making it a promising solution for sustainable and effective wastewater management at the onsite evel.

In the future, our aim is to investigate the reactor's performance under various operating situations and influent characteristics. Furthermore, we will focus our research on optimizing the design and scaling up of the ISBR for broader applications. We intend to contribute to the larger goal of attaining sustainable and efficient wastewater management

practices in both rural and urban settings through constantly improving and advancing this technology.

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References

1. Venkatesh, B.; Velkennedy, R. Formulation of citizen science approach for monitoring Sustainable Development Goal 6: Clean water and sanitation for an Indian city. *Sustain. Dev.* **2023**, *31*, 56–66. [CrossRef]
2. Ritchie, H.; Roser, M. *Clean Water and Sanitation*; OurWorldInData.org: Oxford, UK, 2021.
3. Al Smadi, B.; Al Mhairat, T.; Moqbel, S. Assessment of the Pharmaceutical Waste Management System in Jordan: Regulations and System Characteristics. *J. Hazard. Toxic Radioact. Waste* **2023**, *27*, 05023001. [CrossRef]
4. Murei, A.; Kamika, I.; Samie, A.; Momba, M.N.B. Assessment of the water sources for potential channels of faecal contamination within Vhembe District Municipality using sanitary inspections and hydrogen sulphide test. *Sci. Rep.* **2023**, *13*, 6250. [CrossRef] [PubMed]
5. WHO; UNICEF. *Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baselines*; WHO: Geneva, Switzerland, 2017.
6. Strande, L.; Brdjanovic, D. (Eds.) *Faecal Sludge Management: Systems Approach for Implementation and Operation*; IWA Publishing: London, UK, 2014.
7. Prüss-Ustün, A.; Wolf, J.; Bartram, J.; Clasen, T.; Cumming, O.; Freeman, M.C.; Johnston, R. Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: An updated analysis with a focus on low-and middle-income countries. *Int. J. Hyg. Environ. Health* **2019**, *222*, 765–777. [CrossRef]
8. Conaway, K.; Lebu, S.; Heilferty, K.; Salzberg, A.; Manga, M. On-site sanitation system emptying practices and influential factors in Asian low-and middle-income countries: A Systematic Review. *Hyg. Environ. Health Adv.* **2023**, *6*, 100050. [CrossRef]
9. Pussayanavin, T.; Koottatep, T.; Eamrat, R.; Polprasert, C. Enhanced sludge reduction in septic tanks by increasing temperature. *J. Environ. Sci. Health-Part A Toxic/Hazard. Subst. Environ. Eng.* **2015**, *50*, 81–89. [CrossRef]
10. Lesteur, M.; Bellon-Maurel, V.; Gonzalez, C.; Latrille, E.; Roger, J.; Junqua, G.; Steyer, J.P. Alternative methods for determining anaerobic biodegradability: A review. *Process Biochem.* **2010**, *45*, 431–440. [CrossRef]
11. Smith, A.; Stadler, L.; Love, N.; Skerlos, S.; Raskin, L. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review. *Bioresour. Technol.* **2012**, *122*, 149–159. [CrossRef]
12. Jinturkar, S.B.; Jasud, S.; Jadhav, A.; Biradar, I.N. Improvement of social, economic, and Basic Development in the Backward Village Under 'UNNAT BHARAT ABHIYAAN. *Int. Res. J. Eng. Technol.* **2019**, *6*, 4.

13. Singh, S.; Yadav, P.; Kumar, C.; Sharma, M.; Gaur, R. Performance of an Integrated Settler Based Anaerobic Biofilm Reactor as Onsite Sanitation System. *IETE J. Res.* **2021**, *67*, 603–610. [CrossRef]
14. Singh, S.; Sharma, M.; Kumar, C.; Gaur, R. Effect of Filter Media on Performance of Biofilm Reactor for Treatment of Domestic Wastewater. *J. Hazard. Toxic Radioact. Waste* **2022**, *26*, 04022014. [CrossRef]
15. Sharma, M.; Tyagi, V.; Singh, N.; Singh, S.; Kazmi, A. Sustainable technologies for on-site domestic wastewater treatment: A review with technical approach. *Environ. Dev. Sustain.* **2022**, *24*, 3039–3090. [CrossRef]
16. Truelove, Y.; O'Reilly, K. Making India's cleanest city: Sanitation, intersectionality, and infrastructural violence. *Environ. Plan. E Nat. Space* **2021**, *4*, 718–735. [CrossRef]
17. Singh, S.; Yadav, P.; Kumar, C.; Sharma, M.; Gaur, R. Performance of pilot-scale plant: An integrated settler based anaerobic biofilm reactor for the treatment of domestic wastewater. In Proceedings of the 8th International Conference on Advancements in Engineering and Technology (ICAET-2020), Punjab, India, 20–21 March 2020; pp. 561–566.
18. Kong, Z.; Li, L.; Kurihara, R.; Kubota, K.; Li, Y. Anaerobic treatment of N, N-dimethylformamide-containing wastewater by co-culturing two sources of inoculum. *Water Res.* **2018**, *139*, 228–239. [CrossRef]
19. Li, L.; Qin, Y.; Kong, Z.; Wu, J.; Kubota, K.; Li, Y. Characterization of microbial community and main functional groups of prokaryotes in thermophilic anaerobic co-digestion of food waste and paper waste. *Sci. Total Environ.* **2019**, *652*, 709–717. [CrossRef] [PubMed]
20. Tsushima, I.; Yoochatchaval, W.; Yoshida, H.; Araki, N.; Syutsubo, K. Microbial community structure and population dynamics of granules developed in expanded granular sludge bed (EGSB) reactors for the anaerobic treatment of low-strength wastewater at low temperature. *J. Environ. Sci. Health-Part A Toxic/Hazard. Subst. Environ. Eng.* **2010**, *45*, 754–766. [CrossRef] [PubMed]
21. Lettinga, G. Challenge of psychrophilic anaerobic wastewater treatment. *Trends Biotechnol.* **2001**, *19*, 363–370. [CrossRef]
22. Martin, I.; Pidou, M.; Soares, A.; Judd, S.; Jefferson, B. Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. *Environ. Technol.* **2011**, *32*, 921–932. [CrossRef]
23. Tsegaye, D.; Khan, M.; Leta, S. Optimization of Operating Parameters for Two-Phase Anaerobic Digestion Treating Slaughterhouse Wastewater for Biogas Production: Focus on Hydrolytic–Acidogenic Phase. *Sustainability* **2023**, *15*, 5544. [CrossRef]
24. Arras, W.; Hussain, A.; Hausler, R.; Guiot, S. Mesophilic, thermophilic and hyperthermophilic acidogenic fermentation of food waste in batch: Effect of inoculum source. *Waste Manag.* **2019**, *87*, 279–287. [CrossRef]
25. Kong, Z.; Li, L.; Wang, T.; Rong, C.; Xue, Y.; Zhang, T.; Wu, J.; Li, Y.Y. New insights into the cultivation of N,N-dimethylformamide-degrading methanogenic consortium: A long-term investigation on the variation of prokaryotic community inoculated with activated sludge. *Environ. Res.* **2020**, *182*, 109060. [CrossRef]
26. Aiyuk, S.; Forrez, I.; van Haandel, A.; Verstraete, W. Anaerobic and complementary treatment of domestic sewage in regions with hot climates—A review. *Bioresour. Technol.* **2006**, *97*, 2225–2241. [CrossRef]
27. Noyola, A.; Morgan-Sagastume, J.; López-Hernández, J. Treatment of biogas produced in anaerobic reactors for domestic wastewater: Odor control and energy/resource recovery. *Rev. Environ. Sci. Biotechnol.* **2006**, *5*, 93–114. [CrossRef]
28. Lim, K.; Evans, P.; Parameswaran, P. Long-term performance of a pilot-scale gas-sparged anaerobic membrane bioreactor under ambient temperatures for holistic wastewater treatment. *Environ. Sci. Technol.* **2019**, *53*, 7347–7354. [CrossRef]
29. Yang, Y.; Zang, Y.; Hu, Y.; Wang, X.; Ngo, H. Upflow anaerobic dynamic membrane bioreactor (AnDMBR) for wastewater treatment at room temperature and short HRTs: Process characteristics and practical applicability. *Chem. Eng. J.* **2020**, *383*, 123186. [CrossRef]
30. Schmitt, F.; Banu, R.; Yeom, I.; Do, K. Development of artificial neural networks to predict membrane fouling in an anoxic-aerobic membrane bioreactor treating domestic wastewater. *Biochem. Eng. J.* **2018**, *133*, 47–58. [CrossRef]
31. Maleki, E.; Bokhary, A.; Leung, K.; Liao, B. Long-term performance of a submerged anaerobic membrane bioreactor treating malting wastewater at room temperature (23 ± 1 °C). *J. Environ. Chem. Eng.* **2019**, *7*, 103269. [CrossRef]
32. Vincent, N.; Tong, J.; Yu, D.; Zhang, J.; Wei, Y. Membrane fouling characteristics of a side-stream tubular anaerobic membrane bioreactor (AnMBR) treating domestic wastewater. *Processes* **2018**, *6*, 3–17.
33. Harb, M.; Xiong, Y.; Guest, J.; Amy, G.; Hong, P. Differences in microbial communities and performance between suspended and attached growth anaerobic membrane bioreactors treating synthetic municipal wastewater. *Environ. Sci. Water Res. Technol.* **2015**, *1*, 800–813. [CrossRef]
34. Maleki, E. Psychrophilic anaerobic membrane bioreactor (AnMBR) for treating malting plant wastewater and energy recovery. *J. Water Process Eng.* **2020**, *34*, 101174. [CrossRef]
35. Mei, X.; Wang, Z.; Miao, Y.; Wu, Z. A pilot-scale anaerobic membrane bioreactor under short hydraulic retention time for municipal wastewater treatment: Performance and microbial community identification. *J. Water Reuse Desalination* **2018**, *8*, 58–67. [CrossRef]
36. Ahmed Mekawy, E.; Li, L.; Zhang, Y.; Wang, X. Microbial fuel cells: From fundamentals to applications. A review. *J. Power Sources* **2017**, *356*, 225–244.
37. Dutta, A.; Mukherjee, S.K.; Hossain, S.T. Microbial Fuel Cell Assisted Wastewater Treatment: A Review on Current Trends. In *Microbial Technologies in Industrial Wastewater Treatment*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 59–82.

38. Tang, J.; Yang, H.; Pu, Y.; Hu, Y.; Qu, X.; Chen, S.; Wang, X.C.; Ngo, H.H.; Li, Y.; Abomohra, A. Bioenergy production from swine wastewater based on a combined process of anaerobic dynamic membrane reactor and microalgae cultivation: Feasibility and performance. *Sci. Total Environ.* **2023**, *899*, 165621. [CrossRef] [PubMed]
39. Krishna, G.G.; Kumar, P.; Kumar, P. Treatment of low strength complex wastewater using an anaerobic baffled reactor (ABR). *Bioresour. Technol.* **2008**, *99*, 8193–8200. [CrossRef] [PubMed]
40. Singh, S.; Sharma, M.; Gaur, R. Hydraulic characteristics of integrated settler based biofilm reactor as onsite sanitation system. *Int. J. Chem. React. Eng.* **2021**, *19*, 1317–1325. [CrossRef]
41. Ghosh, A.; Ray, A.; Goswami, A.; Ali, O.A.; Singh, P.K.; Pattnaik, R. Recent Development and Innovations in Integrated Biogas-Wastewater Treatment. In *Biorefinery for Water and Wastewater Treatment*; Springer International Publishing: Cham, Switzerland, 2023; pp. 271–297.
42. Rey-Martínez, N.; Rodríguez-Alegre, R.; You, X.; Martínez-Lozano, S.; Borràs, E.; García-Montaño, J. Assessment of two-stage anaerobic digestion of blackwater and kitchen waste for reducing environmental impact of residential buildings. *Sustain. Chem. Pharm.* **2023**, *33*, 101090. [CrossRef]
43. Singh, S.; Sharma, M.; Gaur, R. Effects of aqwise carrier media and brick media as filter materials on performance of biofilm reactor. *Mater. Today Proc.* **2022**, *60*, 782–787. [CrossRef]
44. Federation, Water Environmental; Aph Association. *Federation, Standard Methods for the Examination of Water and Wastewater*; American Public Health Association (APHA): Washington, DC, USA, 2005.
45. DiLallo, R.; Albertson, O.E. Volatile acids by direct titration. *Water Pollut. Control.* **2012**, *33*, 356–365.
46. Manjunath, P.; Sairam, M.R. Purification and biochemical characterization of three major acidic proteins (BSP-A1, BSP-A2 and BSP-A3) from bovine seminal plasma. *Biochem. J.* **1987**, *241*, 685–692. [CrossRef]
47. Peng, C.; Zhai, Y.; Hornung, A.; Wang, B.; Li, S.; Wang, T.; Li, C.; Zhu, Y. In-depth comparison of morphology, microstructure, and pathway of char derived from sewage sludge and relevant model compounds. *Waste Manag.* **2020**, *102*, 432–440. [CrossRef]
48. Akunna, J.C.; Clark, M. Performance of a granular-bed anaerobic baffled reactor (GRABBR) treating whisky distillery wastewater. *Bioresour. Technol.* **2000**, *74*, 257–261. [CrossRef]
49. Baloch, M.I.; Akunna, J.C. Granular bed baffled reactor (Grabbr): Solution to a two-phase anaerobic digestion system. *J. Environ. Eng.* **2003**, *129*, 1015–1021. [CrossRef]
50. Wang, D.; He, D.; Liu, X.; Xu, Q.; Yang, Q.; Li, X.; Liu, Y.; Wang, Q.; Ni, B.-J.; Li, H. The underlying mechanism of calcium peroxide pretreatment enhancing methane production from anaerobic digestion of waste activated sludge. *Water Res.* **2019**, *164*, 114934. [CrossRef] [PubMed]
51. Nowak, O.; Franz, A.; Svardal, K.; Müller, V.; Kühn, V. Parameter estimation for activated sludge models with the help of mass balances. *Water Sci. Technol.* **1999**, *39*, 113–120. [CrossRef]
52. Goyal, N.K. *Tracing Pollutional Parameters through a UASB Plant, MS Technology Dissertation*; Indian Institute of Technology Roorkee: Roorkee, India, 2003.
53. Amoohadi, V.; Pasalari, H.; Esrafili, A.; Gholami, M.; Farzadkia, M. A comparative study on polyaluminum chloride (PACl) and Moringa oleifera (MO) chemically enhanced primary treatment (CEPT) in enhanced biogas production: Anaerobic digestion performance and the Gompertz model. *RSC Adv.* **2023**, *13*, 17121–17129. [CrossRef]
54. Otieno, E.O.; Kiplimo, R.; Mutwiwa, U. Optimization of anaerobic digestion parameters for biogas production from pineapple wastes co-digested with livestock wastes. *Heliyon* **2023**, *9*, e14041. [CrossRef]
55. Lyu, Z.; Shao, N.; Akinyemi, T.; Whitman, W.B. Methanogenesis. *Curr. Biol.* **2018**, *28*, R727–R732. [CrossRef]

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