

## Article

# Microwave Radiation Influence on Dairy Waste Anaerobic Digestion in a Multi-Section Hybrid Anaerobic Reactor (M-SHAR)

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**Abstract:** Whey is a primary by-product of dairy plants, and one that is often difficult to manage. As whey processing units are costly and complicated, only 15–20% of whey is recycled for use in the food industry. The difficulties in managing waste whey are particularly pronounced for small, local dairy plants. One possible solution to this problem is to use advanced and efficient digesters. The aim of this study was to present an innovative multi-section hybrid anaerobic bioreactor (M-SHAR) design and to identify how microwave radiation heating (MRH) affects methane fermentation of liquid dairy waste (LDW) primarily composed of acid whey. The MRH reactor was found to perform better in terms of COD removal and biogas production compared with the convection-heated reactor. The heating method had a significant differentiating effect at higher organic load rates (OLRs). With OLRs ranging from 15 to 25 kg<sub>COD</sub>·m<sup>-3</sup>·d<sup>-1</sup>, the M-SHAR with MRH ensured a 5% higher COD removal efficiency and 12–20% higher biogas yields.

**Keywords:** liquid dairy waste; whey; anaerobic biodegradation; microwave radiation; anaerobic digestion; methane fermentation; bioreactor



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## 1. Introduction

The dairy industry generates waste that is harmful to the environment by way of its properties and characteristics. High contents of its organic and biogenic compounds are particularly problematic [1,2]. It is estimated that one liter of processed milk can generate up to 10 dm<sup>3</sup> of effluent [3]. Dairy processing plants have a diverse production profile, manufacturing milk, cheeses, yoghurts, butter, and so on, which means that the generated waste varies in composition as well [4,5]. Carbohydrates, proteins, and fats are the predominant constituents, followed by large quantities of residual detergents, acids, and bases from the washing and chemical cleaning process [6]. In addition, such waste has high concentrations of organic nitrogen as well as NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> ions. Its other constituents include phosphorus (in both organic and inorganic form) and the elements Na, K, Ca, Mg, Fe, Co, Ni, and Mn [7].

A primary by-product of dairy plants—one that is often difficult to manage—is whey [8,9]. It is classified into three types based on its pH: acid whey with pH 4.3–4.6, casein whey with pH 4.6–4.7, and sweet (rennet) whey with pH 5.9–6.3 [10]. The most practical method of managing whey is to recycle it for use in the food industry. Whey protein is highly nutritious, with functional properties that are highly desirable for the food industry and widely used in the production of dietary foods for special purposes, as well as high-protein supplements [11]. Whey processing technologies include the following: separation of lactose by nanofiltration; demineralization via ion exchange or via electro dialysis; fractionation using membrane filtration; isolation of constituents via

ion exchange in a fixed-bed bioreactor; isolation of constituents via ion exchange in a moving-bed membrane bioreactor; concentration; evaporation; and drying [12,13].

The high cost of a whey processing unit combined with technological complications means that only 15% to 20% of the waste output is reprocessed and recycled [14]. Waste whey can still be difficult to manage for small, local dairy plants [15]. Releasing non-processed whey into the environment can affect physical and chemical properties of soil and cause degradation of aquatic ecosystems [16,17].

The high organic load of waste whey causes numerous operational problems for treatment systems [18]. Another technological hurdle lies in the high seasonal variance in waste output, which correlates with the volume of milk supply for processing (much higher in summer than winter) [19]. Aerobic processes have proven to be inefficient owing to sludge bulking and excessive biomass growth [20]. They also require high investment costs and a large supply of energy for effluent aeration [21]. As such, anaerobic methods are usually preferred, offering better biodegradation performance for waste with high organic loads [22]. Lactose, the main carbohydrate in dairy waste, is readily metabolized by anaerobic bacteria [23]. Anaerobic reactors can quickly break down casein proteins without producing methane fermentation inhibitors. Anaerobic digestion of lipids is a more complicated process, proceeding in two stages [24]. Firstly, the lipids are hydrolyzed into glycerol and long-chain fatty acids, after which acetate and hydrogen are produced through  $\beta$ -oxidation [25]. Glycerol does not exhibit an inhibitory effect, unlike long-chain fatty acids, which can slow down methane production [26].

Dairy industry waste is neutralized by means of anaerobic methods in multiple types of advanced digesters [27]. These include UASB (upflow anaerobic sludge blanket), ABR (anaerobic baffled reactor), AMBR (anaerobic migrating blanket reactor), ASBR (anaerobic sequencing batch reactor), AnMBR (anaerobic membrane bioreactor), EGSB (expanded granular sludge bed), M-SHFAR (multi-section horizontal flow reactor), and FAF-R (fluidized active filling reactor) [28]. Though many existing methods are available, new improvements to processes are sought to neutralize dairy waste faster and more efficiently while increasing biogas production [28].

Microwave radiation heating (MRH) is one of such potential improvements. Microwave radiation (MR) is selective, meaning that it interacts exclusively with materials having specific dielectric properties, allowing microwaves to penetrate directly into the microbial biomass. MRH is volumetric, and can be stopped immediately by cutting off the power supply [29]. Microwave radiation can induce biological effects depending on the electromagnetic field intensity, wave frequency, wave type, modulation, and exposure duration [30]. Its effects on living organisms are divided into thermal and non-thermal ones [31,32]. The microwave radiation causes a transfer of energy, producing heat, but the outcomes of irradiation also refer to microwave-specific effects that do not result from increased temperature [33]. MR has been noted to affect the structure and function of cellular membranes, promoting genetic differentiation in microorganisms, which translates to an increased efficiency of effluent treatment [34]. Given these advantages, it may be possible—with the right technology—to use microwave radiation to control temperature conditions in anaerobic reactors [35].

The experiments conducted so far have proved a positive influence of the microwave pretreatment-based technologies on the anaerobic digestion effectiveness [36,37]. The highest effectiveness has been observed with methane fermentation of biomass feedstock [38], microalgae [39], wastewater, and sludge [40]. Other studies have also proven the positive influence of the microwaves radiation on the organic substance biodegradation and nutrient removal by active sludge under aerobic conditions [41,42]. Genetic studies have proven an influence of the microwave radiation on bacterial community variety [43,44]. Other investigations confirm the presence of non-thermal effects of microwaves [45,46]. An interesting issue, rarely analyzed, is the use of microwaves for reactors' heating and intensification of biochemical changes in anaerobic digestion.

The aim of this study was to present an innovative multi-section hybrid anaerobic bioreactor (M-SHAR) design and to identify how microwave radiation heating (MRH) affects methane fermentation of liquid dairy waste (LDW) largely composed of acid whey. The study includes an analysis of organic matter biodegradation rates, as well as the volume and qualitative composition of the biogas produced.

## 2. Materials and Methods

### 2.1. Study Design

The present study on LDW methane fermentation was carried out in an innovative M-SHAR. To assess the effect of MRH on methane fermentation, the experiment was separated into two stages with different types of reactor heating: MRH for stage 1 and water-jacket convection heating (WJH) for stage 2. Each stage was grouped into five series according to the organic load rate (OLR) maintained in the M-SHAR, which ranged from 5 to 25 kg<sub>COD</sub>·m<sup>-3</sup>·d<sup>-1</sup>. The methane fermentation was conducted under mesophilic conditions at 40 °C. The duration of the different experimental series varied depending on the hydraulic retention time. In each of the process variants, the M-SHAR minimum operation time was 10 times the time needed for complete exchange of the reactor's active volume. The study design is given in Table 1.

**Table 1.** Study design.

Stage 1—Microwave Heating (MRH)		Stage 2—Convection Heating (WJH)	
Series 1	OLR = 5 kg <sub>COD</sub> ·m <sup>-3</sup> ·d <sup>-1</sup>	HRT = 10.0 days	Q <sub>d</sub> = 10 dm <sup>-3</sup> ·d <sup>-1</sup>
Series 2	OLR = 10 kg <sub>COD</sub> ·m <sup>-3</sup> ·d <sup>-1</sup>	HRT = 5.0 days	Q <sub>d</sub> = 20 dm <sup>-3</sup> ·d <sup>-1</sup>
Series 3	OLR = 15 kg <sub>COD</sub> ·m <sup>-3</sup> ·d <sup>-1</sup>	HRT = 3.3 days	Q <sub>d</sub> = 30 dm <sup>-3</sup> ·d <sup>-1</sup>
Series 4	OLR = 20 kg <sub>COD</sub> ·m <sup>-3</sup> ·d <sup>-1</sup>	HRT = 2.5 days	Q <sub>d</sub> = 40 dm <sup>-3</sup> ·d <sup>-1</sup>
Series 5	OLR = 25 kg <sub>COD</sub> ·m <sup>-3</sup> ·d <sup>-1</sup>	HRT = 2.0 days	Q <sub>d</sub> = 50 dm <sup>-3</sup> ·d <sup>-1</sup>

### 2.2. Materials

The LDW consisted mainly of acid whey, but also contained waste milk, technological effluent from installation washing, and sewage. To achieve the intended OLR in the subsequent experimental series, the daily substrate flow rate was progressively increased. The LDW profile is given in Table 2.

**Table 2.** Profile of the liquid dairy waste (LDW) used in the study.

Parameter	Unit	Value
Chemical Oxygen Demand (COD)	mg <sub>O2</sub> ·dm <sup>-3</sup>	50,000 ± 250
Biological Oxygen Demand (BOD <sub>5</sub> )	mg <sub>O2</sub> ·dm <sup>-3</sup>	32,700 ± 190
Total Nitrogen (TN)	mg <sub>TN</sub> ·dm <sup>-3</sup>	2500 ± 112
Ammonium Nitrogen (AN)	mg <sub>N-NH4</sub> ·dm <sup>-3</sup>	52 ± 7
Total Phosphorus (TP)	mg <sub>TP</sub> ·dm <sup>-3</sup>	520 ± 48
pH	-	7.12 ± 0.12

The study used granular anaerobic sludge sourced from a full-scale dairy effluent treatment plant operated at OLR = 10 kg<sub>COD</sub>·m<sup>-3</sup>·d<sup>-1</sup> and HRT = 24 h. The concentration of inoculum in the M-SHAR was ca. 40 g total solids (TS)·dm<sup>-3</sup>. The anaerobic sludge was loaded into the M-SHAR and filled the lower section. The inoculum was then stabilized over a period of 45 days to adapt to the experimental conditions. The characteristics of the anaerobic inoculum used in the study are given in Table 3.

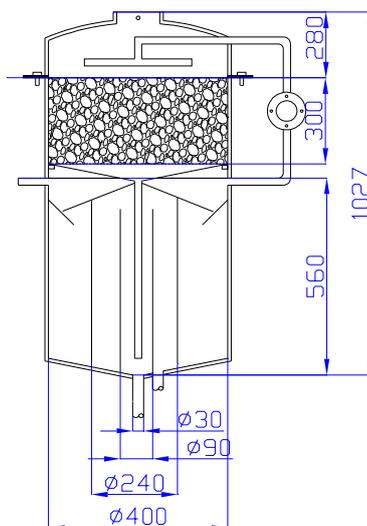
**Table 3.** Characteristics of the anaerobic inoculum used in the study.

Parameter	Unit	Value
Hydration	%	97.8 ± 0.30
Capillary suction time	s	740 ± 24
Total solids	g·dm <sup>-3</sup>	40.1 ± 1.2
Mineral solids	g·dm <sup>-3</sup>	14.4 ± 0.9
Volatile solids	g·dm <sup>-3</sup>	25.7 ± 1.1
Filtrate chemical oxygen demand (COD)	mgO <sub>2</sub> ·dm <sup>-3</sup>	830 ± 31.0
Orthophosphates in filtrate	mgP-PO <sub>4</sub> ·dm <sup>-3</sup>	99.3 ± 16.4
Total nitrogen (TN) in filtrate	mgTN·dm <sup>-3</sup>	148 ± 13.7
Ammonium nitrogen (AN) in filtrate	mgN-NH <sub>4</sub> ·dm <sup>-3</sup>	131 ± 11.9
pH	-	7.49 ± 0.13

### 2.3. Experimental Set-Up

#### 2.3.1. M-SHAR Design

The M-SHAR consisted of a tank with an internal diameter of  $D_C = 400$  mm. The lower section (LS) of the M-SHAR (with the granular sludge) housed two centrally aligned partitions: an inner partition and a central partition to provide a meandering flow of liquid in the lower section. The upper section (US) with the biofilter (PVC, glass transition temperature 80 °C, density 1.28 g·cm<sup>-3</sup>, modulus of elasticity 3000 MPa, long-term use temperature min. -10 °C to max. 70 °C). The main dimensions of the M-SHAR are provided in Figure 1.

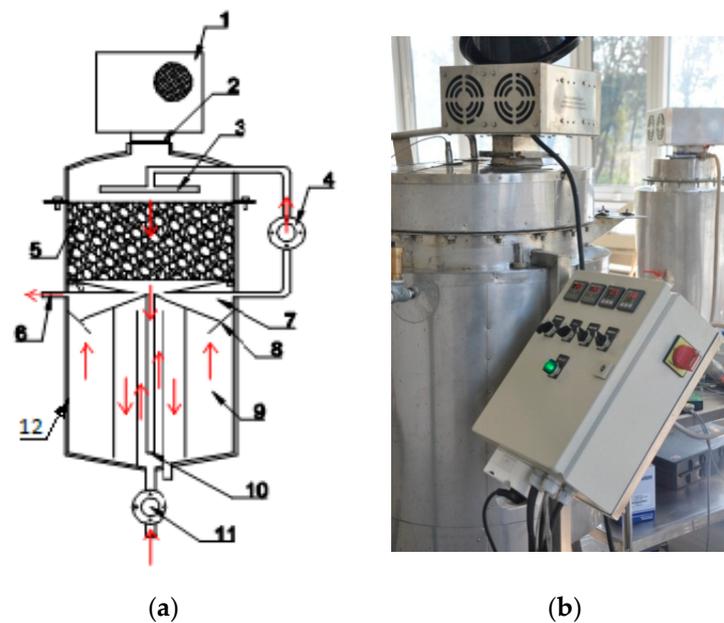


Main dimensions of the M-SHAR:

Total height	$H_c = 1180$ mm
Working height of the LS	$H_{DC} = 560$ mm
Working height of the US	$H_{GW} = 300$ mm
Diameter of the M-SHAR	$D_C = 400$ mm
Working volume of the LS	$V_{DC} = 70$ dm <sup>3</sup>
Working volume of the US	$V_{GC} = 30$ dm <sup>3</sup>
Total working volume	$V_{CC} = 100$ dm <sup>3</sup>

**Figure 1.** Main dimensions of the multi-section hybrid anaerobic reactor (M-SHAR).

The LS outlet was blocked by a baffle with a gap  $B_s = 3$  mm in width. Above the gap was a reactor outlet with a water seal and a suction nozzle for the pump recirculating the wastewater to the US (with an immobilized anaerobic biosludge bed). The filling of the US was set on a steel grid, located above a reversed cone. The narrow tip of the reversed cone fed into a central hose with a diameter of  $D_R = 2.0$  cm, the end of which was located 4.7 cm above the raw wastewater inlet of the lower section (Figure 2a). All structural components of the reactor were made of acid-proof steel (Figure 2b). The housing was made of a 2.0 mm thick steel sheet.



**Figure 2.** Diagram (a) and photograph (b) of the M-SHAR (1—microwave generator, 2—microwave generator seal, 3—LDW supply, 4—circulation pump, 5—US of bed, 6—outlet, 7—LS; sludge separation area, 8—sludge collector, 9—LS; granular sludge area, 10—central hose, 11—LDW metering pump, 12—water jacket used in stage 2).

### 2.3.2. M-SHAR Functioning Principles

LDW was fed from the bottom of the reactor with an Allweiler ASL 10 pump at a constant flow (Figure 2). The feed rate ranged from  $10 \text{ dm}^3 \cdot \text{d}^{-1}$  to  $50 \text{ dm}^3 \cdot \text{d}^{-1}$ , depending on the series. The treated LDW drained from the granular sludge section via a nozzle fitted halfway through the height of the reactor (Figure 2). A meandering flow of liquid was maintained in the granular sludge section: the wastewater flowed up, then downwards in the middle section, and up again through the outer partition into the outlet connector (Figure 2). The upper (granular sludge-filled) part was equipped with a sludge collector. The collector worked by limiting the flow of liquid via a conical baffle that formed a single system with the outer baffle. The combined baffles left only a 5 mm gap for the liquid to flow through (Figure 2). Above the collector baffle, outside of the outlet, was a suction inlet for the pump used to recirculate the liquid over the filling. The pump was an Allweiler ASL 10. The recirculated liquid was percolated through the filling placed on the support grate, on which the anaerobic biofilm was grown. After flowing through the bed layer, the wastewater was fed into a collecting funnel under the support grate, then discharged through a hose to the bottom of the granular sludge section (Figure 1).

### 2.3.3. Heating System

Microwave radiation was generated by a 1.6 kW magnetron at a frequency of 2.45 GHz. The complete microwave heating system was manufactured by Plazmatronika NT<sup>®</sup>, Wrocław, Poland. The magnetron operated at an efficiency of 52%. The magnetron on/off times were regulated by a temperature controller (two four-channel HI 98801 thermometers, Hanna Instruments, Póvoa de Varzim, Portugal). The microwave generator was located above the filling. MR was introduced to the M-SHAR interior with a wave-guide. A seal was fitted between the M-SHAR and the magnetron, allowing passage of the incoming MR without letting any biogas out. The seal was made of PVC, a material transparent to MR.

The control reactor was heated with a water jacket. To provide optimal temperature, a  $40 \text{ dm}^3$  tank for water with an electric heater was used (2000 W). The temperature inside the reactor was monitored and switched the circulating pump (capacity  $4 \text{ m}^3 \cdot \text{h}^{-1}$ ) in the water jacket. The entire reactor was insulated with a 50 mm layer of mineral wool. The

temperature sensor for the control of the water circulation pump was located at the bottom filling layer.

#### 2.3.4. Reactor Filling

The US of the reactor was filled with plastic carriers. The carriers were plastic balls, 30 mm in diameter, with grooves to increase the surface area (Figure 3). The filling parameters were as follows: total volume  $V_t = 30.0 \text{ dm}^3$ , surface area  $F_a = 0.1256 \text{ m}^2$ , specific surface area  $s = 315 \text{ m}^2 \cdot \text{m}^{-3}$ , single ball (carrier) diameter  $R = 30 \text{ mm}$ , tongue width  $B_t = 1 \text{ mm}$ , and groove width  $B_g = 1 \text{ mm}$ .



**Figure 3.** Filling of the US (with anaerobic biofilm).

#### 2.4. Analytical and Statistical Methods

The temperature inside the reactors was constantly monitored. Temperature changes were recorded simultaneously in the upper and lower sections of the reactor, at four points each. The temperature was measured and recorded via two four-channel HI 98801 thermometers (Hanna Instruments, Póvoa de Varzim, Portugal). At each of the eight measurement points, temperature readouts were performed once every 60 min. Biogas output was measured using an Aalborg mass flow meter equipped with an instantaneous flow display and a totalizer. Biogas quality was assessed using a GC Agilent 7890 A gas chromatograph. Methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) levels were monitored as well. The influent and effluent were analyzed once a day for COD using cuvette tests and a DR 2800 spectrophotometer with a mineralizer (HACH Lange, Düsseldorf, Germany). The VSS was determined gravimetrically (part E of EPA Standard Method 2540). The pH was measured using a VWR 1000  $\text{dm}^3$  pH-meter. The titration method (Tritlab AT 1000, Hach, Düsseldorf, Germany) was used to determine the FOS/TAC ratio (volatile organic acid and buffer capacity ratio). The measurements were done once a day.

The Statistica 13.1 PL software package (StatSoft, Inc., Tulsa, OK, USA) was used for the analysis. Homogeneity of variance in groups was determined using a Levene's test. Tukey's HSD test was applied to determine the significance of differences between the series ( $p = 0.05$ ).

### 3. Results and Discussion

The LDW consisted mainly of acid whey, but also contained waste milk, technological effluent from installation washing, and sewage. COD concentration of about  $50 \text{ g} \cdot \text{dm}^{-3}$  was claimed. In the research of Carvalho et al. (2013), a COD range of  $50\text{--}102 \text{ g} \cdot \text{dm}^{-3}$  was observed [47]. A high variability in LDW was presented in Escalante et al. (2017) experiments, where COD ranged between  $65$  and  $140 \text{ g} \cdot \text{dm}^{-3}$  [48]. In the Mainardis et al. (2019) [49] and Karadag et al. (2015) [50] studies, the concentration of COD was  $79 \text{ g} \cdot \text{dm}^{-3}$  and  $60\text{--}68.6 \text{ g} \cdot \text{dm}^{-3}$ , respectively. Moreover, the BOD/COD ratio was shown to be above 0.5, indicating an easily degradable substrate [51].

The experiment showed that the efficiency of organic compound biodegradation was mostly determined by the OLR. The COD removal efficiency significantly decreased as OLR increased in the successive series. The highest efficiencies (more than 80%) were noted in series 1 and 2 for both heating variants (Figure 4). Incremental decreases in LDW biodegradation efficiency were observed in series 4, with 65% COD removal for stage 1 and 59% for stage 2 (Figure 4). The organic removal performance was the poorest in series

5 (less than 50%) (Figure 4). MRH was found to significantly affect COD removal within the OLR range of  $15 \text{ kg}_{\text{COD}} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  to  $25 \text{ kg}_{\text{COD}} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ . In the series within this range, the MRH showed 5–6% higher LDW treatment performance than the WJH. The viability of treating dairy effluent with anaerobic beds was examined by Omil et al. (2003), who achieved near 90% COD removal at an OLR of  $5\text{--}6 \text{ kg}_{\text{COD}} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ . Reducing HRT from 20 to 3 days did not produce significant changes in treatment performance [52]. Extremely high COD removal efficiency (98%) was achieved at  $\text{OLR} = 9.8 \text{ kg}_{\text{COD}} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  by Mendez et al. (1989). However, the use of an anaerobic filter for neutralizing whey necessitated a very long HRT of 142 days [53].

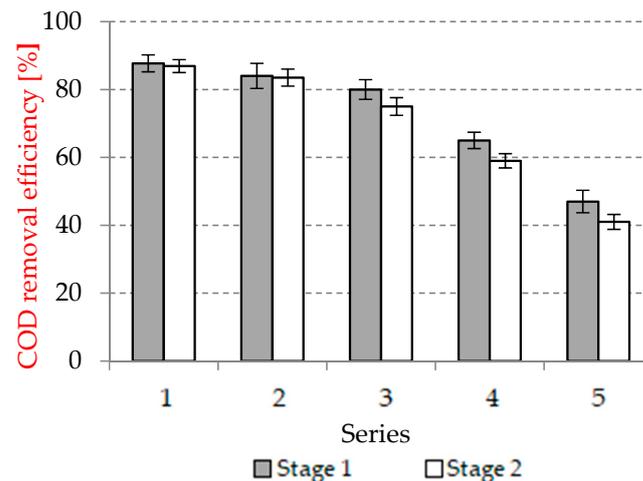


Figure 4. COD removal efficiency.

Organic load removal was directly linked to the treatment performance and the OLR. The highest COD removal rate of over  $1000 \text{ g}_{\text{COD}} \cdot \text{d}^{-1}$  was observed for series 3–5, where the OLR ranged between  $15$  and  $25 \text{ kg}_{\text{COD}} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  (Figure 5). In the case of series 4 and 5, the values were significantly higher in stage 1, with COD removal rates almost  $120 \text{ g}_{\text{COD}} \cdot \text{d}^{-1}$  higher than in stage 2 (Figure 5). Series 1 performed the worst for both heating types, with COD removal rates approximating  $440 \text{ g}_{\text{COD}} \cdot \text{d}^{-1}$  (Figure 5). The OLR levels employed in various studies have varied greatly. For example, Najafpour et al. (2008) tested OLRs ranging from  $7.9$  to  $45.42 \text{ kg}_{\text{COD}} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ , achieving 98% organic matter removal from whey at  $35 \text{ }^\circ\text{C}$  [54]. Out of the many anaerobic reactor types covered in the literature, hybrid reactors are the closest match for the design used in the present study. They usually combine a UASB design with an anaerobic filter bed. Ramasamy et al. (2004) used such a design to degrade synthetic effluent with a COD of  $10 \text{ g} \cdot \text{dm}^{-3}$ . The organic load rate ranged from  $0.82$  to  $6.11 \text{ kg}_{\text{COD}} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ , and the hydraulic retention time from  $4.1$  to  $1.7 \text{ d}$ . With these process parameters, and under mesophilic conditions, COD removal efficiencies varied between 90% and 97% [55].

The highest biogas production was achieved in series 3, where the OLR was maintained at  $15 \text{ g}_{\text{COD}} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$ . The yields for this process variant averaged  $433 \text{ dm}^3_{\text{biogas}} \cdot \text{d}^{-1}$  in stage 1 and  $384 \text{ dm}^3_{\text{biogas}} \cdot \text{d}^{-1}$  in stage 2 (Figure 6). As was the case for series 4 and 5, the difference in daily biogas yields between these stages was statistically significant ( $p = 0.05$ ). The poorest biogas production of less than  $180 \text{ dm}^3_{\text{biogas}} \cdot \text{d}^{-1}$  was observed for series 1 and, in this case, no significant discrepancies in biogas yields were observed between the two reactor heating types (Figure 6). With an OLR of  $10 \text{ g}_{\text{COD}} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$  in series 2, the biogas production was quite stable and close to  $317 \text{ dm}^3_{\text{biogas}} \cdot \text{d}^{-1}$ , regardless of the M-SHAR heating used (Figure 6).

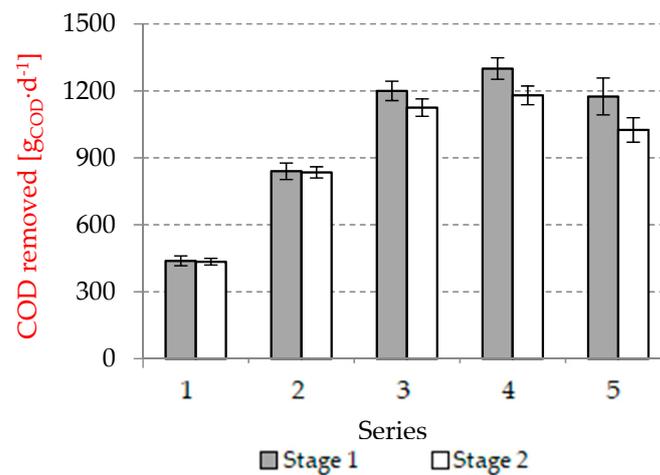


Figure 5. Daily COD removed.

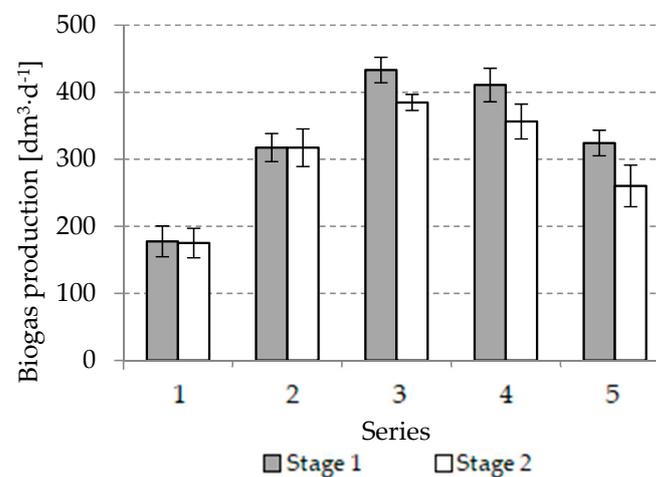


Figure 6. Daily biogas production.

Literature reports indicate that living organisms exposed to the unique environment of electromagnetic microwave fields are subject to various non-thermal effects [56,57]. Banik et al. (2003) showed that *Methanosarcina barkeri* DS-804 exposed to microwave radiation had higher colony counts and cell sizes. The experiment used a pure culture exposed to microwave at frequencies between 13.5 and 36.5 GHz for 2.0 h. The composition of the biogas produced by a pure *Methanosarcina barkeri* DS-804 culture changed depending on the microwave frequency. The methane content in the examined biogas reached values as high as 76.5% at a frequency of 31.5 GHz, and as low as 52.3% in the biogas produced by the control cultures, not exposed to microwave radiation. According to the authors, these findings indicate that microwaves induce specific metabolic activity that correlates with faster growth rates [58].

Another significant parameter used to measure fermentation performance is the biogas production coefficient, which describes the biogas yield per organic load removed. Its values were the highest in series 1 for both experimental stages, exceeding  $400 \text{ dm}^3 \cdot \text{kgCOD}_{\text{removed}}^{-1} \cdot \text{d}^{-1}$  (Figure 7). The experiments revealed a linear correlation between higher OLRs and daily biogas production, the latter of which fell below  $300 \text{ dm}^3 \cdot \text{kgCOD}_{\text{removed}}^{-1} \cdot \text{d}^{-1}$  in series 5 (Figure 7). Statistically significant differences in the biogas production coefficient were only found at  $\text{OLR} = 15 \text{ gCOD} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$  in series 3, i.e.,  $361 \text{ dm}^3 \cdot \text{kgCOD}_{\text{removed}}^{-1} \cdot \text{d}^{-1}$  and  $342 \text{ dm}^3 \cdot \text{kgCOD}_{\text{removed}}^{-1} \cdot \text{d}^{-1}$  for stage 1 and stage 2, respectively (Figure 7).

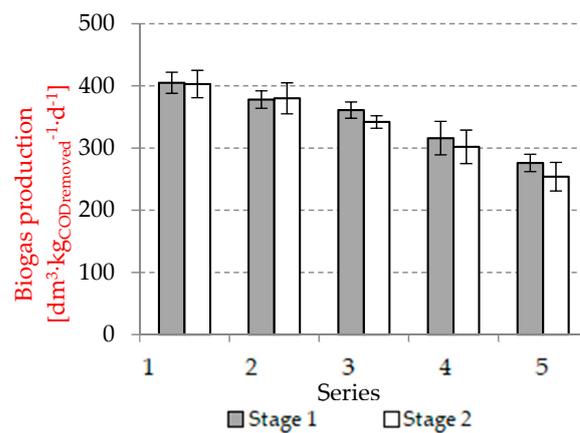


Figure 7. Daily biogas production per COD load removed.

The methane content of the biogas was directly linked to the OLR and decreased successively with the increase in OLR. In contrast, it was not found to be significantly affected by the heating method used. The methane fraction peaked in series 1 at almost 73% (Figure 8). In turn, a significantly reduced level of methane in biogas (below 50%) was recorded in series 4, where the reactor load was  $20 \text{ gCOD} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$  in series 4 (Figure 8).

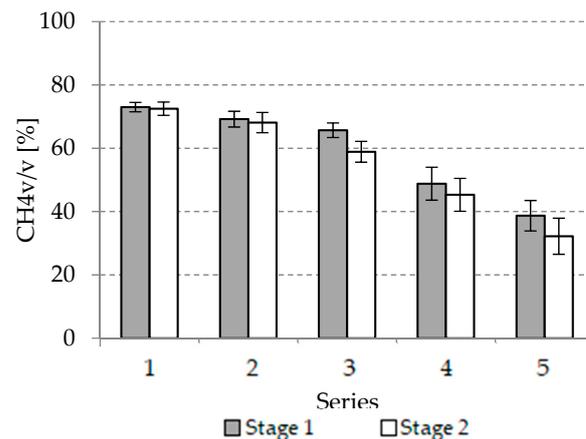


Figure 8. Methane fraction in the biogas.

In assessing how the presented reactor design has performed, an important aspect to consider is the concentration of suspended solids in the reactor effluent. If the amount of outflowing biomass was to exceed the growth in the reactor, it would result in biomass leaching and reduced performance. The design of the sludge separator was identical in both reactors, and the choice of the heating method had no effect on the levels of suspended solids in the outflow. These levels were similarly low in series 1–3, ranging between  $0.54 \text{ g}_{\text{DM}} \cdot \text{dm}^{-3}$  and  $0.912 \text{ g}_{\text{DM}} \cdot \text{dm}^{-3}$  (Figure 9). Series 4 produced significantly higher values of ca.  $1.60 \text{ g}_{\text{DM}} \cdot \text{dm}^{-3}$ , while the peak levels of suspended solids were recorded in series 5—around  $3.8 \text{ g}_{\text{DM}} \cdot \text{dm}^{-3}$  (Figure 9).

The effect of microwave radiation on biochemical processes has not yet been fully explored. Parker et al. (1996), investigating the effect of microwaves on the activity of hydrated lipase, found that the rate of enzymatic reaction for the irradiated enzyme was 2–3 times higher than in the conventionally heated system [59]. According to Dahl et al. (2007), most enzymes are inactivated as a result of rapid temperature fluctuations under microwave radiation. Nevertheless, immobilization was found to promote enzymatic transesterification of (R,S)-2-octanol and non-thermal effects of microwave radiation [60]. Other researchers have also noted that immobilization improves enzyme stability in microwave fields [61,62].

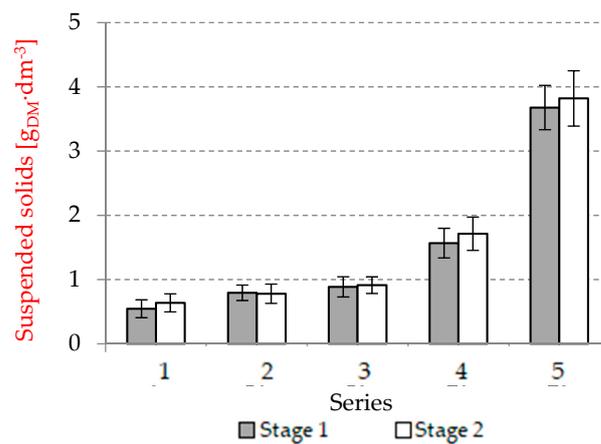


Figure 9. Concentration of suspended solids in the effluent.

A study by Cydzik-Kwiatkowska et al. (2012) on the effect of microwaves on anaerobic biofilm found improved biodiversity after microwave exposure. The Shannon–Wiener index for a conventionally-heated submerged bed reactor was 1.74, but increased to 2.53 when microwave heating was employed. The  $H'$  index for trickling-bed reactors was 1.74 (conventional heating) and 1.93 (microwave heating) [63].

The higher tolerance of the microwave-treated biomass to deteriorating environmental conditions may be attributable to differences in population structure. A FISH assay was performed to establish the abundance of specific Archaea—namely those of the family *Methanosarcinaceae* and genus *Methanosaeta*—as a percentage of total cells in the sampled biomass [64]. Two acetotrophic families of the order *Methanosarcinales* were counted. *Methanosarcina* have the most adaptable metabolism among the methanogenic organisms, whereas *Methanosaeta* can only produce methane from acetate. The inoculum sludge contained  $14 \pm 2.8\%$  *Methanosarcinaceae* and  $9.9 \pm 4.7\%$  *Methanosaeta*. The *Methanosarcinaceae* accounted for 3.2 to 7.2% of all micro-organisms across the experimental series, with the fraction being significantly higher in the microwave-heated series. The *Methanosaeta* fraction totaled  $1.4 \pm 1\%$  in the control series at a temperature of 35 °C and a load of 1 kg COD·m<sup>-3</sup>·d<sup>-1</sup>, and was completely non-existent in the other experimental variants [64]. Table 4 presents the results of pH measurements and the values of the FOS/TAC ratio.

Table 4. pH and FOS/TAC values depending on the variant of the experiment.

Series	OLR [kgCOD·m <sup>-3</sup> ·d <sup>-1</sup> ]	Stage 1—Microwave Heating (MRH)		Stage 2—Convection Heating (WJH)	
		pH	FOS/TAC	pH	FOS/TAC
1	5	7.29 ± 0.11	0.32 ± 0.03	7.27 ± 0.06	0.30 ± 0.01
2	10	7.21 ± 0.08	0.34 ± 0.02	7.24 ± 0.09	0.33 ± 0.01
3	15	7.02 ± 0.06	0.35 ± 0.04	6.99 ± 0.13	0.37 ± 0.04
4	20	6.82 ± 0.12	0.43 ± 0.05	6.74 ± 0.04	0.47 ± 0.06
5	25	6.57 ± 0.10	0.54 ± 0.03	6.49 ± 0.08	0.55 ± 0.02

Significantly decreased effluent treatment and methane fermentation efficiencies were correlated with an observed pH decrease to  $6.82 \pm 0.12$  and FOS/TAC ratio increase to  $0.43 \pm 0.05$  in series 3. Han et al. (2001) [65] stated that neutral pH ensures optimal conditions for anaerobic processes. Chavarria et al. (2018) stated that methanogenic bacteria are inhibited in conditions of pH lower than 6.7 [66]. The optimum FOS/TAC ratio for anaerobic digestion stands between 0.3 and 0.4, in which the biogas production is at a maximum. A lower FOS/TAC ratio indicates that the micro-organisms in the system are starving and the digester should be fed with more substrate, whereas a higher FOS/TAC ratio denotes that the system is overfed [67]. When the OLR is too large, total volatile fatty acids (TVFAs) accumulate when the production rate in the system is higher than the

consumption rate, resulting in an acid inhibition problem [68]. Then, the pH value and the CH<sub>4</sub> volumetric yield decrease significantly [69]. Kazimierowicz et al. (2021) stated a significant influence of OLR on the methane fermentation efficiency of food waste products. It was found that OLR above 6 kg ODM·m<sup>-3</sup>·d<sup>-1</sup> completely inhibited the process [70]. Cheng et al. (2018) found that OLRs have little adverse effect on effluent quality and organic matter removal efficiency, with an increase of OLR to 9.72 kg<sub>COD</sub>·m<sup>-3</sup>·d<sup>-1</sup>. However, when the OLR was further increased to 14.58 kg<sub>COD</sub>·m<sup>-3</sup>·d<sup>-1</sup>, the biogas production rate decreased significantly to 1.35 dm<sup>3</sup>·dm<sup>-3</sup>·d<sup>-1</sup>. COD, carbohydrate, and protein concentration in the effluent increased greatly, accompanied by a sharp decrease in the removal efficiency [71]. Sánchez et al. (2005) also proved that raising OLR may cause the increase in volumetric methane production, but also may contribute to the risk of system failure due to accumulation of VFAs and free ammonia in the system, as well as pH decrease [72].

High ammonia concentrations increase the pH, which inhibits the anaerobic digestion process [73]. Total ammonium nitrogen (TAN) is the sum of free ammonia nitrogen (FAN) and NH<sub>4</sub><sup>+</sup>. In fermentation reactors, the forms of ammonia nitrogen can transform. High pH and temperature stimulate the conversion to FAN, which is the most toxic form of ammonia nitrogen [74]. It has the ability to penetrate the cell membrane of fermenting microorganisms, which ultimately leads to the inhibition of enzymatic activity [73]. Toxic concentrations of ammonium nitrogen depend on many technological and environmental factors, ranging from 53 mg·dm<sup>-3</sup> to 1450 mg·dm<sup>-3</sup> (FAN) and 1500 to 7000 mg·dm<sup>-3</sup> (TAN) [68].

In order to obtain in-depth knowledge on technological efficiency and obtain reliable and confirmed data related to investment and operating costs, it is necessary to conduct continuous studies on a pilot or semi-technical scale. An important and required stage is to raise the technology readiness level (TRL) to a higher stage. The effectiveness of the technology has to be proven in work in its final form and under expected conditions. This can be reliably verified in TRL 8. In almost all cases, this TRL represents the end of true system development. Examples include developmental test and evaluations of the system in its intended weapon system to determine if it meets design specifications. In order to obtain full and confirmed knowledge on both technological and economic aspects, it is necessary to build and ensure long-term operation of the installation in real conditions.

#### 4. Conclusions

The heating method was found to have an impact on M-SHAR performance, with the MRH reactor performing better in terms of organic load removal and biogas production compared with the WJH. The effect of the heating method was significant at higher OLRs. The M-SHAR with MRH ensured a 5% higher COD removal efficiency and 12–20% higher biogas yields when OLR was maintained at 15 to 25 kg COD·m<sup>-3</sup>·d<sup>-1</sup>. However, it should be noted that methane content in the biogas was significantly determined by the OLR and was not affected by the heating method used. The present study indicates that MRH improves the stability and efficiency of methane fermentation at higher OLRs.

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