water

MDPI

Article

Effect of pH, COD, and HRT on the Performance of Microbial Fuel Cell Using Synthetic Dairy Wastewater

Aritro Banerjee *, Rajnish Kaur Calay and Subhashis Das

Faculty of Engineering Science and Technology, UiT-The Arctic University of Norway, 8514 Narvik, Norway; rajnish.k.calay@uit.no (R.K.C.); subhashis.das@uit.no (S.D.)
* Correspondence: aritro.banerjee@uit.no

Abstract: Microbial fuel cells (MFC) are emerging technologies that can produce electricity while treating wastewater. A series of tests were carried out to evaluate the efficiency of this technology for treating dairy wastewater (DWW). The experiments used *Shewanella baltica* as an exoelectrogen in a small single MFC to treat simulated DWW. The impacts of various operational factors, specifically pH, hydraulic retention time (HRT), and chemical oxygen demand (COD) in the influent to the anode chamber, were investigated, and the effect of these variables on the output performance of the cell was evaluated. The best performance of the MFC was found when the pH, HRT, and COD were 8, 6.66 h, and 20,632 mg/L, respectively, in the scaled experimental setup. Under these conditions, the maximum power density and percentage removal of COD in terms of wastewater treatment ability were found to be 138 mW/m^2 and 71%, respectively. It may be concluded that MFCs are suitable treatment technologies for treating dairy wastewater while potentially simultaneously generating power.

Keywords: microbial fuel cell; dairy wastewater; *Shewanella baltica*; sodium lactate; facultative anaerobic bacteria

check for updates

Citation: Banerjee, A.; Calay, R.K.; Das, S. Effect of pH, COD, and HRT on the Performance of Microbial Fuel Cell Using Synthetic Dairy Wastewater. *Water* **2023**, *15*, 3472. https://doi.org/10.3390/w15193472

Academic Editor: Abasiofiok Mark Ibekwe

Received: 31 August 2023
Revised: 22 September 2023
Accepted: 27 September 2023
Published: 30 September 2023

1. Introduction

The dairy industry substantially impacts the amount and characteristics of total wastewater discharge, especially in developed countries. Each liter of pasteurized milk generates approximately twice as much effluent water [1]. The quantity of effluent generated by dairy businesses depends on process enhancements and adherence to regulations. In addition, the logistics of transporting dairy products require routine cleansing. While water use standards for cleansing have been established, due to a lack of regulations, the volume of wastewater generated often exceeds that of production factories [2–4].

The composition of DWW comprises total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD), carbonaceous materials, casein, and proteins that can be converted to carbonaceous materials [5,6]. It usually also contains residual nitric acid and sodium hydroxide because staff facilities such as restrooms and showers contribute effluents comparable to municipal sewage wastewater, increasing the overall COD, BOD, and TSS in DWW [6]. The characteristics of dairy effluent vary depending on industrial processes and variables such as pH, volumetric flow rate, and temperature.

The many operational factors affecting wastewater quality in the dairy industry vary from one another [7], as shown in Figure 1.

The quantity of wastewater from the dairy industry largely depends on standard operating procedures and good manufacturing practices. Due to the modifications that have been made to the process of making milk and dairy products over the years, it has been measured that the volume of wastewater generation can be controlled and decreased from 2.5 times to 1 per liter of milk production [8]. This indicates that the changes in operations and processes in the dairy industry have led to the minimization of the volume of dairy wastewater per liter of dairy products [9]. The purest water, which can be reused for irrigation or factory washing, is produced during the pasteurization stage of milk preparation [10]. Various pasteurization procedures have specific temperature requirements. In addition, milk processing reactors and apparatus require routine cleaning, resulting in wastewater containing residual nitric acid and sodium hydroxide [11,12]. Staff restrooms and showers contribute effluents comparable to municipal sewage to industrial dairy effluents [13]. Sanitary wastewater increases COD, BOD, and TSS to some extent [14,15]. Aerobic and anaerobic biological treatments are traditionally used to treat dairy wastewater [16]. A research study showed that separating sanitary waste from dairy effluents reduces treatment costs by separating pathogens and microbes [12,17]. However, if a Microbial Fuel Cell (MFC) is introduced to the treatment scheme, there is no need to separate the sanitary waste from the DWW at the facility [18]. The mixed culture present in the sanitary waste from the dairy industry lowers treatment costs and benefits microbial fuel cell performance (MFCs) [19–21].

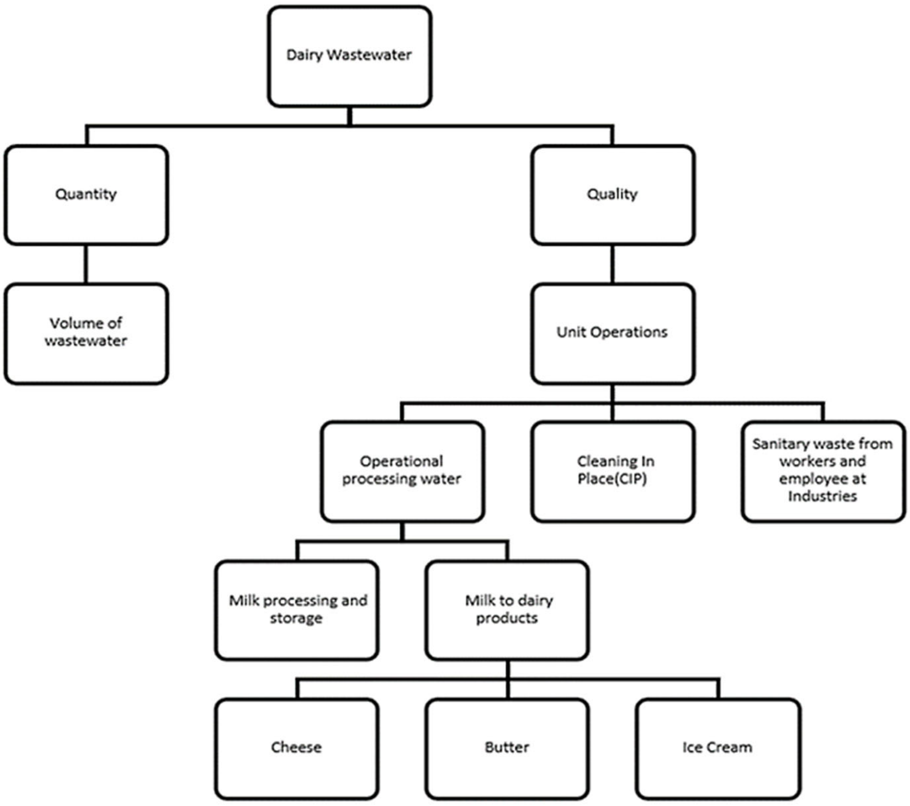


Figure 1. Classification of dairy wastewater.

Traditional dairy effluent treatment methods involve multiple unit operations, including expensive secondary biological treatment [22]. The biological treatment process includes aerobic and anaerobic treatment processes.

Aerobic treatment requires a sequencing batch reactor (SBR) [23], activated sludge process (ASP) [24], trickling filters, etc. [25,26]. In anaerobic treatment, anaerobic digestors, up-flow anaerobic sludge blanket reactors, etc., are essential [27]. These different biological treatment methods have many issues, as shown in Figure 2. Due to their ability to combine wastewater remediation with bioelectricity generation, microbial fuel cells (MFCs) offer a promising approach. MFCs convert chemical potential directly into electricity, rendering them economical, sustainable, and environmentally favorable [28].

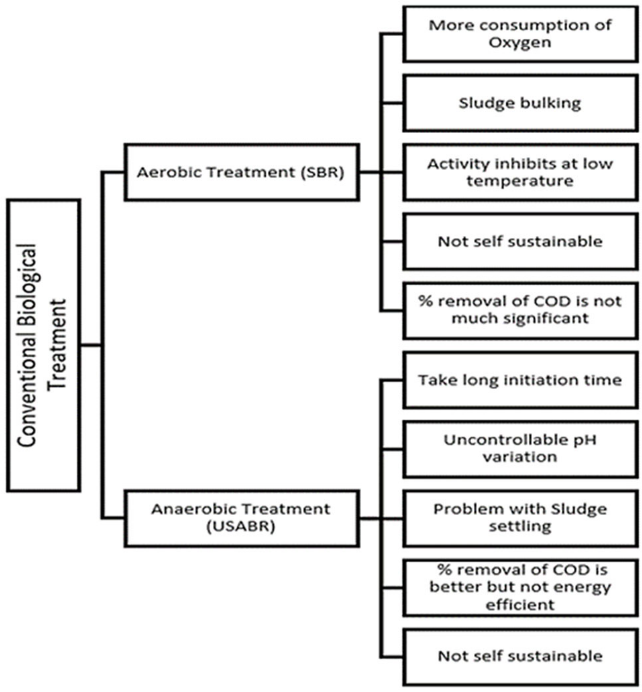


Figure 2. The drawbacks of the traditional biological treatment of DWW [9].

Lactose and casein found in dairy wastewater increase the organic effluent load. Generally, whey has 4–5% lactose and 1% protein [29]; thus, the leakage and trace amount of such a dairy product like whey in utility or cleaning water can contribute to a high organic load with an increase in the COD, BOD, and TSS of dairy utility water (cooling tower or boiler) and dairy wastewater respectively [5,30–32]. Under anaerobic conditions, lactose decomposition produces lactic acid, which lowers pH and oxygen levels, potentially endangering aquatic ecosystems [33–35]. MFCs are essential in removing nitrogenous organic compounds and reducing the organic load in dairy wastewater. Utilizing mixed cultures as exoelectrogens in MFCs improves their performance, especially with sanitary waste. Therefore, the use of MFCs in dairy wastewater plays a significant role in some specific exoelectrogens. In dairy processing, sugar fermentation contributes to the formation of lactic acid, while caustic soda solution is commonly used for degreasing dairy equipment [36]. Using such a degreasing reagent leads to sodium lactate formation, as shown in Equation (1) [37], one of the most important substrates for exoelectrogens in MFCs.

$$\begin{array}{c} CH_3CHOHCOOH + NaOH \;\rightarrow\; CH_3CHOHCOONa + H_2O \\ (Lactic\ Acid)\ (Sodium\ Hydroxide)\ (Sodium\ Lactate) \end{array} \tag{1}$$

Shewanella baltica is a facultatively anaerobic exoelectrogen that can compete both aerobically and anaerobically [38–40]. Using facultative anaerobic exoelectrogens in MFCs can prove more practically viable in large-scale operations. The species is also capable of forming a biofilm on the surface of electrodes and is considered an exoelectrogen [41]. The *Shewanella* species can also have a role in the three electron transfer mechanisms: direct, indirect, and mediator [42,43]. In the absence of electron acceptors, the cell wall of *Shewanella* species experiences structural changes, leading to the formation of nanowires [44]. This species can direct electron transfer and secretes flavin [45], a yellow-pigmented molecule that reduces metals in the effluent. Due to its extracellular metal reduction potential [46], *Shewanella* species can be suitable for bioremediation and numerous applications in MFCs, including biofuel production [40], biosensors [47], and CO_2 fixation [48].

The pH of dairy effluent varies considerably depending on the production of different dairy products. Wastewater from dairy facilities producing whey, cheese, etc., is typically acidic, whereas wastewater from milk and cream products has a pH closer to neutral. Due to the use of detergent and degreasing chemicals, producing a large quantity of butter increases the alkalinity of dairy wastewater. The range of pH of wastewater from various dairy products is shown in Figure 3.

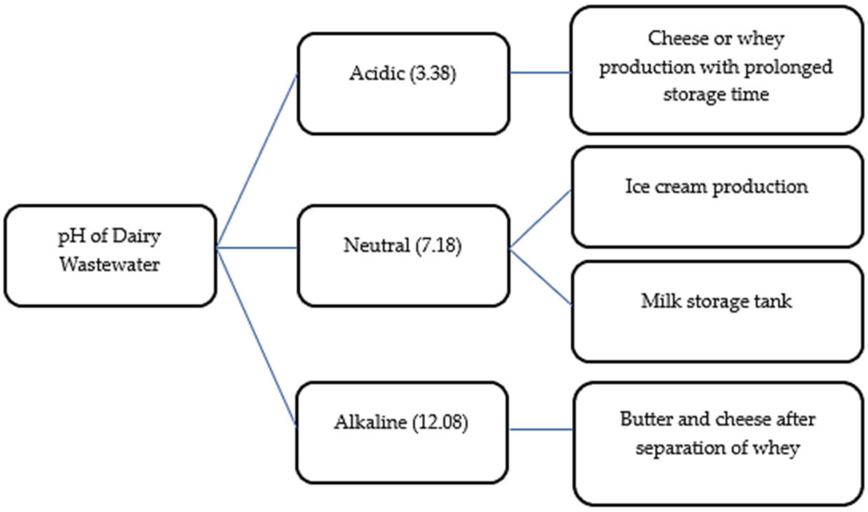


Figure 3. pH of wastewater from different dairy products [9,32,49–52].

As an exoelectrogen in microbial fuel cells (MFCs), *S. baltica* can compete in the presence and absence of oxygen. The pH of effluent water varies significantly based on the quantity of the various dairy products produced. Nonetheless, excessively acidic or alkaline wastewater conditions may inhibit microbial activity in the anode chamber. MFC efficacy is determined by the rate and kinetics of the biochemical reactions taking place in the anode chamber. Therefore, it is essential to optimize the pH of dairy wastewater as an input parameter to improve the output performance of MFCs.

Depending on the quantity of the various dairy products produced, the concentration of carbonaceous substances in dairy wastewater, as measured by COD, varies considerably. The presence of organic matter influences the output efficacy of MFCs significantly. Low levels of organic matter in wastewater result in weak MFC performance. However, increasing organic matter concentration does not always result in improved performance. Each microorganism has an optimal substrate concentration, above which substrate inhibition occurs, creating a suffocating environment for microorganisms. Optimizing the substrate concentration is essential for enhancing the efficacy of MFCs. If dairy effluent contains an excessive organic load that exceeds the optimized value, system performance must be maintained by applying the necessary treatments. The range of COD of the wastewater from various dairy products is presented in Figure 4 [9,32,49–53].

A series of experiments are necessary to comprehend the growth kinetics of *S. baltica*. To evaluate microbial growth kinetics, four distinct batch mode operations of *S. baltica* were conducted by modulating the sodium lactate concentration in the media for a particular design of MFC. The maximum specific growth rate (μ_{max}) obtained was 0.83 h^{-1}, indicating that the hydraulic retention time (HRT) for the system in continuous mode should not surpass 0.138 mL/min [54]. When the COD of the synthetic wastewater was 10,272 mg/L and the feed wastewater flow rate was maintained at 0.1 mL/min, the obtained power density was approximately 12 mW/m^2. Under these conditions, the maximum COD removal percentage achieved about 57%. The output performance of an MFC largely depends on the HRT [55–57]. It is noticeable that the maximum power density value achieved from the experiment is not significant. To enhance the performance of MFC, HRT should be increased to some extent so that it can be operated under continuous mode with a lower feed flow rate [54].

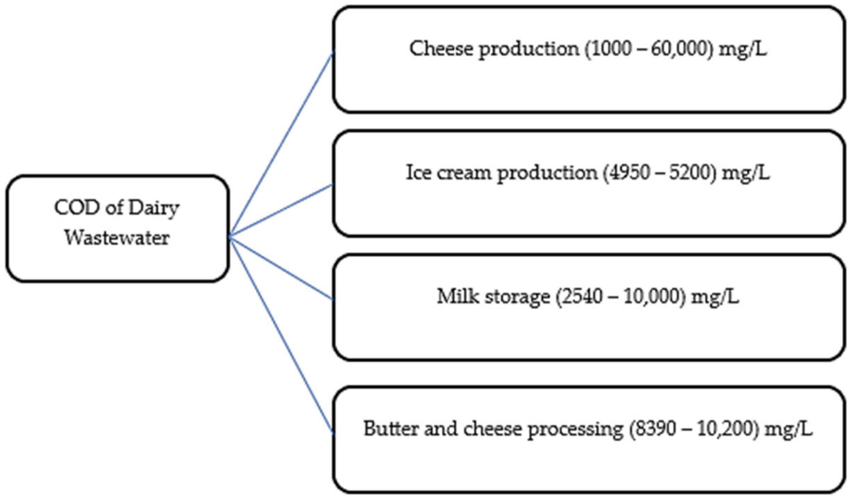


Figure 4. COD of wastewater from different dairy products [9].

Numerous research studies have been conducted to investigate how MFC technology can manage various types of wastewater with the modification of electrodes and membranes [58–61]. Some papers describe the application of MFC technology in the dairy and food industries [62,63]. One study found that regulating the pH and temperature of *Saccharomyces cerevisiae* as an exoelectrogen in dairy effluent improved the output of MFC in terms of power generation and water treatment [64]. Another study reported MFC-treated wastewater from various unit activities of cheese-producing companies with COD removal [65]. Lactate is a viable substrate compared to acetate for *Shewanella* species [66], but there has not been much research into employing *Shewanella baltica* as an exoelectrogen in dairy effluent. Thus, for this paper, we set out to experimentally investigate the MFC technology for treating dairy wastewater by using *Shewanella baltica* as an exoelectrogen. For this purpose, a single MFC was built, and synthetic dairy wastewater was used. The variation of pH and the organic matter, often expressed as COD, of dairy wastewater impacts the performance when an MFC is introduced into a treatment process. The fluctuation of such parameters is primarily due to the variation in the manufacturing quantity of different dairy products, as explained in Figures 1, 3, and 4. The flow rate of the dairy wastewater treatment plant was designed based on the quantity and quality of wastewater produced. In the present research study, we describe the inlet condition of dairy wastewater that is suitable for MFC to treat and generate energy using *Shewanella baltica*. Therefore, three critical operating parameters, i.e., pH, substrate concentration, and HRT, were studied and optimized to maximize the performance of the system in terms of both percentage removal of COD and power output.

2. Materials and Methods

2.1. Experimental Setup

The MFC was made out of two identical chambers, anode and cathode, with a volume of 9 mL each by using plexiglass plastic. The dimensions of these chambers were $3 \times 4 \times 0.75$ cm^3. The wastewater was fed into the anode chamber through an inlet nozzle and taken out through an outlet nozzle. The inlet and outlet nozzles of the cathode chambers were kept open for natural air circulation. Nafion 117 (Sigma Aldrich, St. Louis, MO, USA) [67] was used to separate the anode and cathode chambers. Two carbon felts with dimensions of $4 \times 5 \times 0.02$ cm^3 were used as electrodes. The entire set up and the experiment was performed in The Miljø Laboratory at at UiT, The Arctic University of Norway, Narvik.

Two solid plexiglass plastic plates supported or held the defragment parts together. These two solid plates are called end plates, and they have four holes at four corners to fit M5 bolts. The anode and cathode chambers were placed in between these two end plates.

M5 butterfly nuts were used to join all the components and sandwiched between the two end plates as shown in Figure 5. Rubber gaskets were used between each component to make the system leakproof. Copper wire was used to connect the two carbon felt electrodes placed in the anode and cathode chambers.

Figure 5. Plate-type MFC unit used for our experiment showing the three separate components (cathode, membrane, anode) in between two end plates.

2.2. Wastewater Composition

Synthetic dairy wastewater was prepared by adding the components shown in Table 1 to one liter of distilled water.

Table 1. Composition of synthetic dairy wastewaters [54,55].

Components	Concentration
Ammonium Chloride (NH_4Cl)	40 mg/L
Magnesium Chloride ($MgCl_2$)	10 mg/L
Copper(II) Sulfate ($CuSO_4$)	0.1 mg/L
Calcium Chloride ($CaCl_2$)	5.0 mg/L
Manganese(II) Sulfate ($MnSO_4$)	0.1 mg/L
Zinc Chloride ($ZnCl_2$)	0.1 mg/L
Phosphate buffer	1 mol/L
Potassium Chloride (KCl)	60 mg/L
Magnesium Sulfate ($MgSO_4$)	50 mg/L
Ferric Chloride ($FeCl_3$)	3.0 mg/L
Nido Milk powder	2500 mg/L

Sodium lactate was used and varied to alter the initial concentration of the substrate from 0.2 to 3% w/w [68]. Based on a previous research work, the pH of the water was changed by using HCl and NaOH solutions (5–9).

2.3. Exoelectrogen

The facultative anaerobic bacteria, *S. baltica*, was chosen as a Gram-negative exoelectrogen. The bacterial strain was isolated and provided by the SINTEF Narvik AS. Initially, *S. baltica* was allowed to grow in 100 mL LB broth media at 25 °C for 48 h [54]. In order to ensure that the *S. baltica* acclimatized, 10 mL from the 100 mL inoculated media was taken out and added into the anode chamber for 5–7 days. This led to the formation of a biofilm on the surface of the anode [69]. A batch study was carried out simultaneously at different substrate concentrations by using a Monod model to determine the maximum specific growth rate and substrate saturation constant. This study further helped set the hydraulic retention time of feeding of DWW under continuous mode operation [54].

2.4. Instruments

A Masterflex® peristaltic pump (Metrohm AG, Herisau, Switzerland) with a variable flow rate was continuously fed DWW in the anode chamber. The DR-3900 spectrophotometer (Hach Instruments) was used to evaluate the COD of DWW in the Miljø Laboratory at UiT, The Arctic University of Norway, Narvik. A digital pH meter was used to determine the pH values of the DWW at different points. Pico data logger was used to determine the real-time voltage and current during the operation.

3. Experimental Procedure

A total of 10 mL from the inoculum was fed into the anode chamber to form a biofilm on the surface of the anode under anaerobic conditions. During this phase of the procedure, the MFC worked as a batch process. The anode and cathode were connected to the data logger to measure the open circuit potential (OCP). Concerning the previous research work, a stable voltage was obtained after ten days of batch mode operation [54]. The stable voltage is an indicative way of predicting biofilm formation. Biofilm formation was enhanced in the absence or lack of sufficient substrate concentrations [70–72].

The synthetic dairy wastewater was fed into the anode chamber under the continuous mode of operation by using a peristaltic pump (Masterflex Metrohm) once biofilm formation was completed by monitoring stable OCP. The produced biofilm on the surface of the anode in the anode chamber converted the lactic acid in the wastewater into a different form of carbon compounds, electrons, and protons. The electrons were made to flow through an external circuit, and protons migrated through Nafion 117 to the cathode chamber. The electrons flowed through an external circuit and produced electricity. Based on previous studies, lactic acid can be decomposed in three possible ways with three different chemical reactions. All three possible reactions form electrons, protons, and other organic compounds such as pyruvate, acetate, and carbon dioxide. Assuming the complete decomposition of lactic acid into carbon dioxide, the reaction can be expressed as shown in Equation (2). Lactic acid can be decomposed into pyruvate and acetate, as described in Equations (3) and (4) [73]. The experimental study was study in three different flow rates (0.025, 0.05, and 0.1 mL/min). The initial CODs were varied in each flow rate from 2242 to 31,112 mg/L. In each substrate concentration, the pH was varied from 5 to 9 in order to evaluate the performance of the MFC in terms of the percentage removal of COD and power generation.

$$CH_3CHOHCOOH + 3H_2O \rightarrow 3CO_2 + 12H^+ + 12e^- \quad (2)$$

$$CH_3CHOHCOOH \rightarrow CH_3COCOOH + 2H^+ + 2e^- \quad (3)$$

$$CH_3CHOHCOO + H_2O \rightarrow CH_3COO + CO_2 + 5H^+ + 5e^- \quad (4)$$

The samples from the outlet of synthetic DWW in the anode chamber were collected periodically to analyze the COD and pH for 60 different sets of operations. Polarization curves were obtained from each and every individual experiment by connecting two electrodes with variable external loads. The resistance of the external loads varied from 40 to 0.1 kohm. During this phase, the voltages were constantly monitored in the data logger. The current generated was calculated by using Ohm's law. The currents produced at different voltages were calculated per unit area of electrode in terms of current densities by using Equations (5) and (6). The power generation at different external loads was computed using Equation (7). The percentage removal of CODs at different inlet conditions was determined by using Equation (8).

$$V = IR \tag{5}$$

$$I_d = I/m^2 \tag{6}$$

$$P_d = VI/m^2 \tag{7}$$

$$\%\ removal\ of\ COD = 100 \times \frac{COD_{in} - COD_{out}}{COD_{in}} \tag{8}$$

The maximum power density and the current density were analyzed online based on the polarization curves for each individual set of experiments [74].

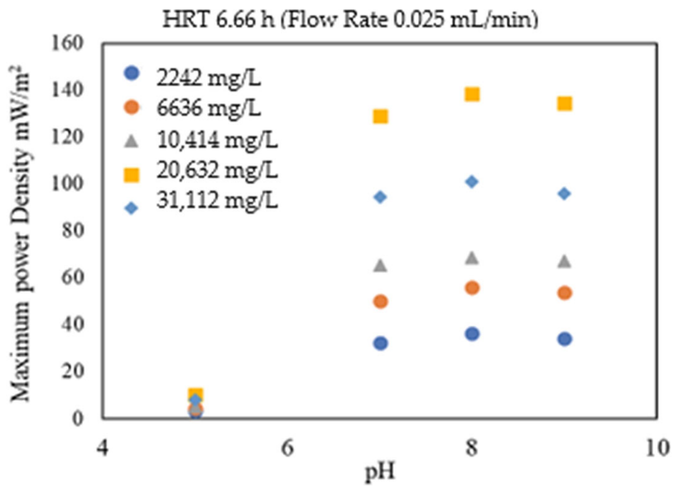
4. Results and Discussion

4.1. The Effect of Initial CODs on Output Performance

The initial COD of the dairy wastewater varied from 2242 to 31,112 mg/L. It was observed that with an increase in the initial concentration of the substrate, the output performance of MFC increased; however, non-significant power generation was observed when further increasing the concentration beyond 20,632 mg/L. Beyond this concentration, the COD removal rate was also limited and followed the same curve pattern for all ranges of pH and HRT, as shown in Figure 6a–f.

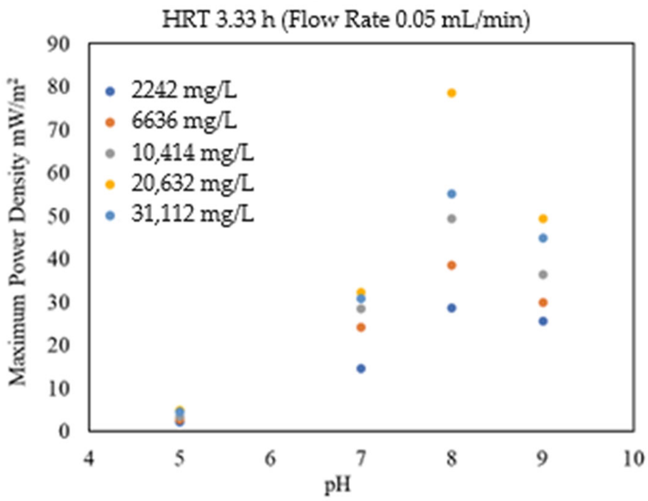
This drastic decrease in power generation can be attributed to the self-inhibition of the growth of *S. baltica* under a huge carbon loading rate [75]. At a substrate concentration of 31,112 mg/L, no more exoelectrogen can be present for substrate degradation. Therefore, excess substrate accumulates and affects the microbial activity of *S. baltica*.

Beyond 20,616 mg/L of initial COD, excess biofilm formation on the surface of the anode can worsen the performance of the MFC. Excess biofilm formation may clog the system, resulting in a higher internal resistance, which restricts the mass transfer in the anode chamber.

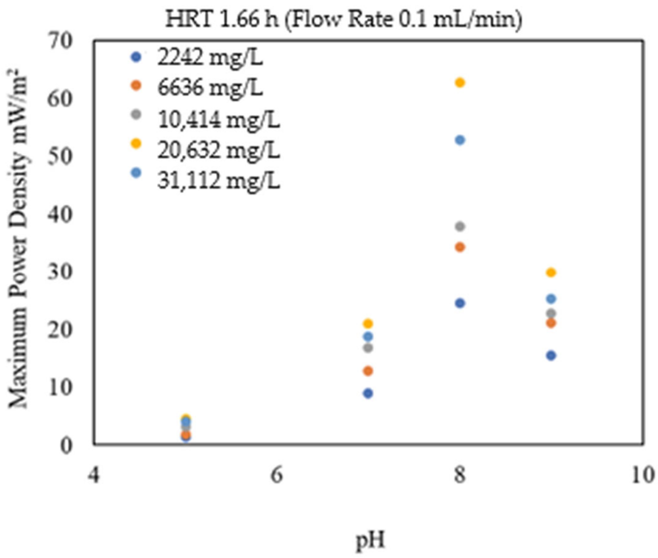


(**a**)

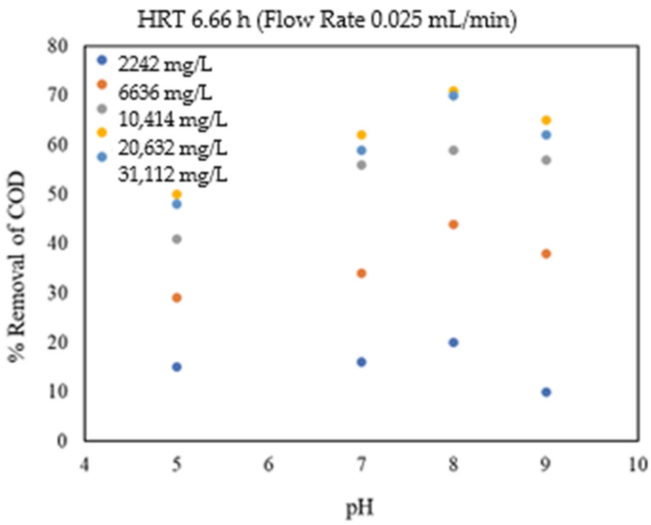
Figure 6. *Cont.*



(**b**)

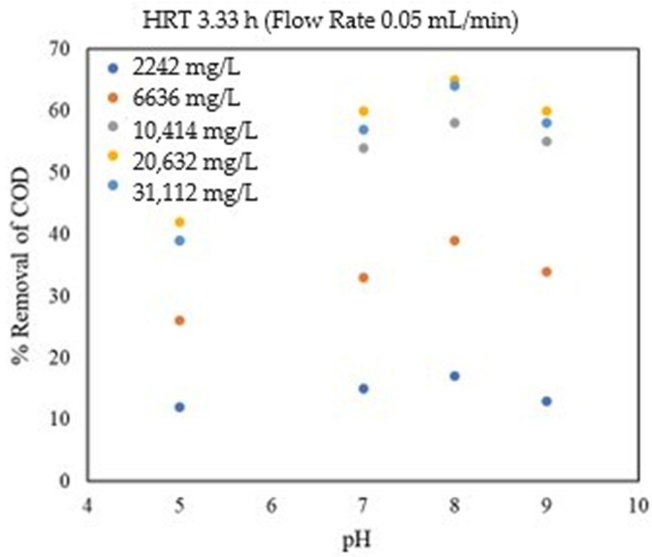


(**c**)

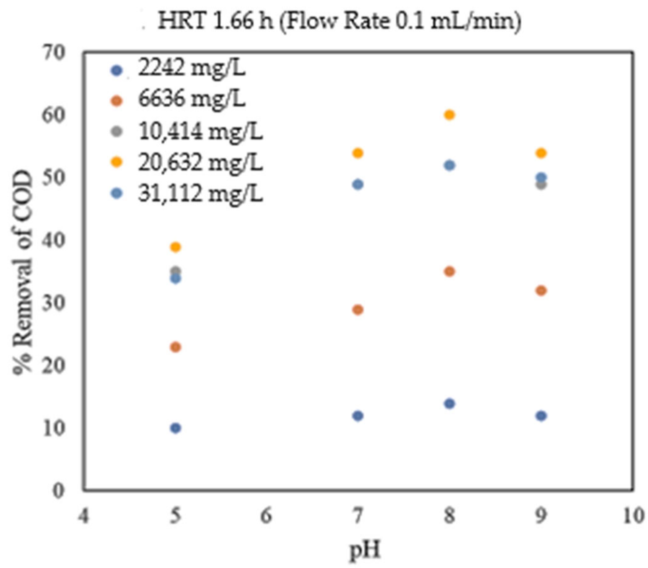


(**d**)

Figure 6. *Cont.*



(**e**)



(**f**)

Figure 6. Maximum power densities of MFC at different initial CODs and pH values (**a**) with HRT of 6.66 h, (**b**) with HRT of 3.33 h, (**c**) with HRT of 1.66 h. Maximum percentage removal of COD at different initial CODs and pH values (**d**) with HRT of 6.66 h, (**e**) with HRT of 3.33 h, (**f**) with HRT of 1.66 h.

A surplus substrate can inhibit the oxidation mechanism in the anode chamber and also alter the food per microorganism ratio (F/M). This ratio signifies the food to microorganism initial COD per unit of time divided by the number of microorganisms present in the volatile suspended solid [74]. Generally, the F/M ratio is specific to a particular microorganism and ranges from 0.4 to 0.5 gCOD/gVSS for MFCs. For this reason, the rate of COD removal can also be decreased under such conditions. Therefore, diluting the dairy wastewater is recommended if the water contains a higher substrate concentration (above 20,632 mg/L). This dilution has been shown to lead to higher MFC output performance by optimizing the initial COD for this particular system.

4.2. The Effect of pH on Output Performance

The inlet pH of feed DWW varied from 5 to 9, and the maximum power density was obtained when the pH was set at 8. An increase or a decrease in pH value from 8 leads to a decline in the power generation and percentage removal of COD and follows the same curve pattern for all ranges of COD and HRT, as shown in Figure 6a–f.

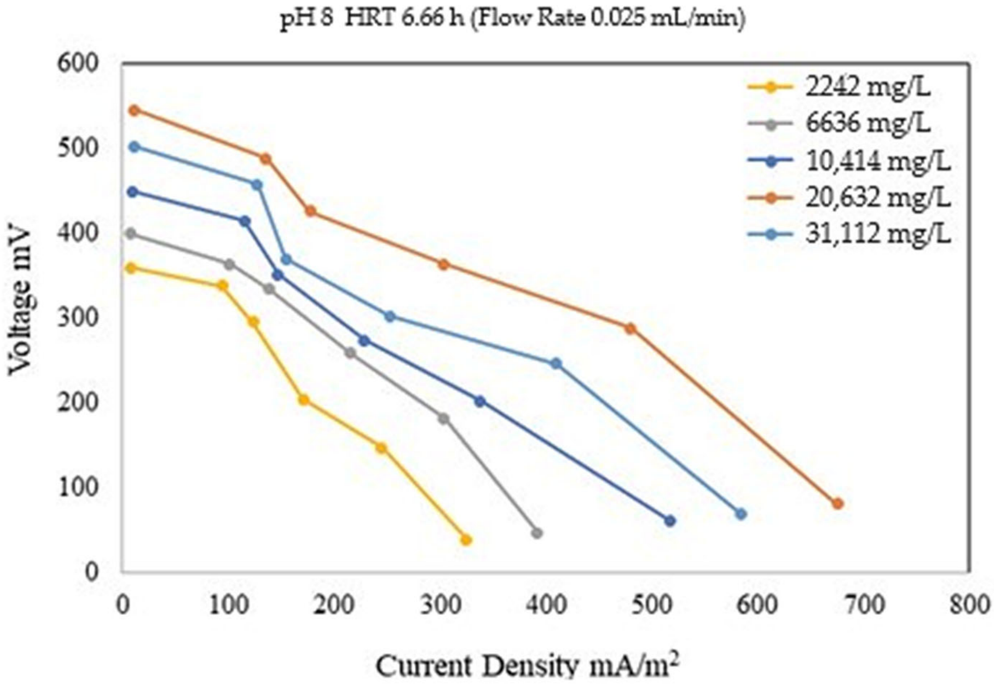
Operating the MFC at a pH of 5 led to the activation of the acidogenic bacteria. Under this condition, the production of the H_2 mechanism is more dominant over the degradation of carbonaceous organic matter [76]. Such a condition leads to a decrease in the COD removal rate compared to neutral and higher pH values. Therefore, the low pH value of the fed DWW is no longer suitable for both power generation and wastewater treatment. At neutral pH (around 7), the methanogenic bacterial activity was more favorable than the splitting up of organic matter into protons and electrons [77]. Therefore, fewer electrons were produced at pH 7, resulting in lower power generation. However, the COD removal rate was still found to be noticeably better at neutral pH compared to the acidic environment. When the pH value of the fed DWW was around 8, a significant increase in the power generation and COD removal rate was observed. Under this condition, the activities of methanogenic and acidogenic bacteria are suppressed [78]. The activity of the exoelectrogen was found to be dominant when the pH value was set to 8.

When the pH value increased from 8 to 9, indicating a more alkaline condition, a sudden drop in power generation was observed, and the percentage removal of COD also dropped. It is assumed that the microbial activity of *S. baltica* might have been inhibited when the pH was around 9. A batch study is recommended to observe the growth kinetics of *S. baltica* within an alkaline environment [79].

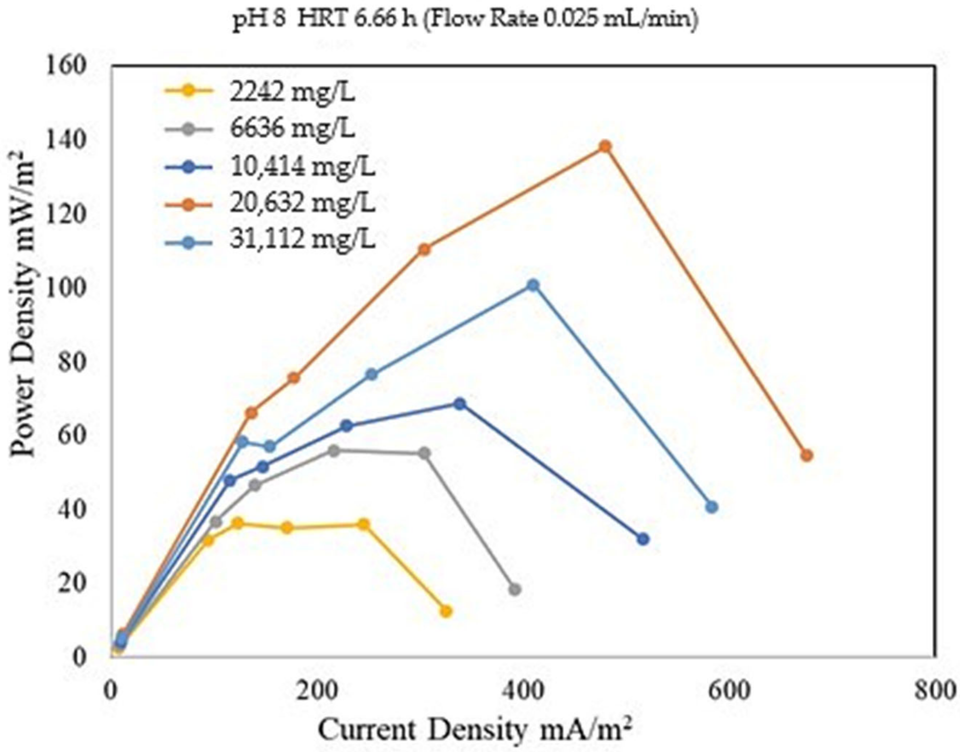
4.3. The Effect of HRT on Output Performance

The HRT of the system varied from 1.66 to 6.66 h by changing the feed flow rate of wastewater from 0.1 to 0.025 mL/min. The output performance of the MFC is dependent on the feed flow rate. At a higher feed flow rate, which corresponds to a lower HRT, the performance of the MFC decreases. We also performed a batch study of *Shewanella baltica*, and the observed value of μ_{max} was 0.83 h^{-1}. This value indicates that, in order for the current system design to avoid washout conditions, the MFC cannot be operated above a flow rate of 0.14 mL/min [54]. The maximum power density was obtained at a flow rate of 0.025 mL/min, corresponding to an HRT of 6.66 h. Considering the volume of the MFC, a further decrease in the feed flow rate can tend the system towards batch mode operation. An increase in feed flow rate value from 0.025 mL/min leads to a decline in the power generation and percentage removal of COD and follows the same curve pattern for all ranges of initial COD and pH, as shown in Figure 6a–f.

The polarization curve of the MFC at the optimized pH, HRT, and initial COD values, which were 8, 6.66 h, and 20,632 mg/L, is shown in Figure 7a. It represents the voltage monitored by the data logger when the MFC was fed with DWW at different closed circuit resistance ranges (from 40 to 0.1 Kohm). The current density was calculated using Equation (3) for every voltage. Figure 7b shows the maximum power density obtained at optimum conditions as a function of current density; this was calculated by using Equation (4). The maximum percentage of COD removal in the dairy wastewater at 6.66 h HRT and with an initial COD of 20,632 mg/L is plotted against different pH values in Figure 8, and it was calculated using Equation (5).



(**a**)



(**b**)

Figure 7. Polarization curve of MFC under optimum pH and HRT at different Initial CODs (**a**) cell voltage as a function of current density (**b**) output power density as a function of current density.

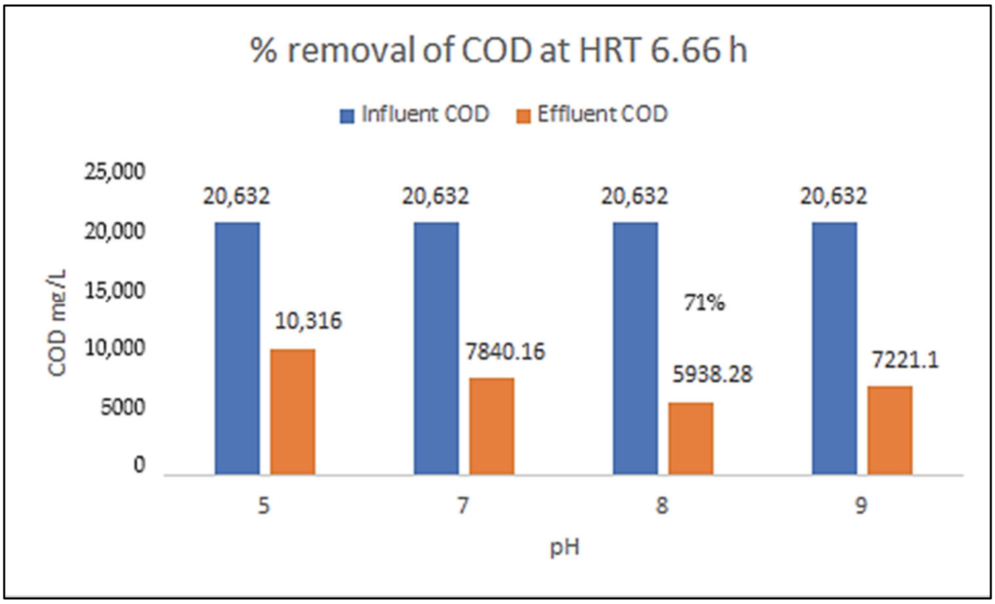


Figure 8. The percentage removal of COD with HRT 6.66 h and initial COD of 20,632 mg/L at different pH.

5. Conclusions

The present study used a single MFC and *S. baltica* to treat DWW. The study highlighted the significant parameters that influence the performance of an MFC. From our study, the following conclusions may be made:

- HRT is an essential factor that affects the biochemical reactions within an MFC, which is controlled by the flow of the feed wastewater. HRT needs to be optimized for different MFC designs and sizes. For this case study, an HRT of 6.66 h yielded the best performance, which was achieved by maintaining a flow rate of 0.025 mL/min. Flow rates higher than 0.14 mL/min lead to washout conditions within the MFC [54].
- The pH of dairy wastewater varies between 4 and 10. The results of our experiment showed better performance using *S. baltica* at pH 8 compared to other pH values.
- The initial COD indicates the limitation of handling organic load for any MFC system. In the present study, a feed water COD of 20,632 mg/L was found to be the maximum organic load for the operation of the MFC. A higher value of organic load inhibits the performance of the MFC. Therefore, prior analysis of wastewater quality is necessary to adjust the organic load to obtain the desired output from the system.
- The best MFC performance in terms of power generation and % removal of COD was observed at a pH value of 8, a HRT of 6.66 h, and an initial COD concentration of 20,632 mg/L, with values of 138.21 mW/m^2 and 71%, respectively.

This study presents the performance results of a single MFC, one simulated wastewater type, and a specific exoelectrogen. The effects of pH, initial COD concentration, and flow rate on the performance of the MFC were experimentally analyzed, leading to a better way of approaching the use of MFC. However, in order to facilitate the real application of MFCs in wastewater treatment, system scaling up is required in order to use the produced energy [80]. The produced energy can be used to measure the biological oxygen demand (BOD) of the wastewater as a biosensor. Therefore, MFCs can simultaneously provide a sustainable system for treating and sensing the quality of dairy wastewater. Scaling up a system involves stacking single cells to enhance the COD removal rate and simultaneous power generation. However, cell stacking requires design optimizations based on the hydrodynamic flow distribution of the wastewater and electrical connections of the circuits to achieve the maximum COD removal efficiency and electric output, respectively.

Author Contributions: Conceptualization, A.B. and S.D.; methodology, A.B.; validation, A.B. and R.K.C.; formal analysis, A.B. and S.D.; data curation, A.B. and S.D.; writing—original draft preparation, A.B.; writing—review and editing, R.K.C. and S.D.; supervision, R.K.C.; project administration, R.K.C.; funding acquisition, R.K.C. All authors have read and agreed to the published version of the manuscript.

Funding: The work was carried out for PhD research, and UiT funded the APC.

Data Availability Statement: Not Applicable (All the data associated with the paper has been mentioned within the manuscript).

Conflicts of Interest: The authors declare that they do not have any competing interests or any kind of personal relationships that could affect the work shown in this article.

References

1. Bouamra-Mechemache, Z.; Réquillart, V.; Soregaroli, C.; Trévisiol, A. Demand for dairy products in the EU. *Food Policy* **2008**, *33*, 644–656. [CrossRef]
2. Rankin, S.A.; Bradley, R.L.; Miller, G.; Mildenhall, K.B. A 100-Year Review: A century of dairy processing advancements—Pasteurization, cleaning and sanitation, and sanitary equipment design. *J. Dairy Sci.* **2017**, *100*, 9903–9915. [CrossRef] [PubMed]
3. Ranieri, M.L.; Huck, J.R.; Sonnen, M.; Barbano, D.M.; Boor, K.J. High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk. *J. Dairy Sci.* **2009**, *92*, 4823–4832. [CrossRef] [PubMed]
4. Suozzo, A. Pasteurization and Its Discontents: Raw Milk, Risk, and the Reshaping of the Dairy Industry. 2015, p. 111. Available online: https://scholarworks.uvm.edu/graddis/320/ (accessed on 20 April 2023).
5. Kolev Slavov, A. Dairy Wastewaters—General Characteristics and Treatment Possibilities—A Review. *Food Technol. Biotechnol.* **2017**, *55*, 2017. [CrossRef] [PubMed]
6. Kohle, A.S.; Pawar, V.P. Physico-chemical analysis of effluents from dairy industry. *J. Recent Res. Sci. Technol.* **2011**, *3*, 29–32.
7. Sar, T.; Harirchi, S.; Ramezani, M.; Bulkan, G.; Akbas, M.Y.; Pandey, A.; Taherzadeh, M.J. Potential utilization of dairy industries by-products and wastes through microbial processes: A critical review. *Sci. Total Environ.* **2022**, *810*, 152253. [CrossRef] [PubMed]
8. Stasinakis, A.S.; Charalambous, P.; Vyrides, I. Dairy wastewater management in EU: Produced amounts, existing legislation, applied treatment processes and future challenges. *J. Environ. Manag.* **2022**, *303*, 114152. [CrossRef] [PubMed]
9. Ganta, A.; Bashir, Y.; Das, S. Dairy Wastewater as a Potential Feedstock for Valuable Production with Concurrent Wastewater Treatment through Microbial Electrochemical Technologies. *Energies* **2022**, *15*, 9084. [CrossRef]
10. Kolhe, A.S.; Ingale, S.R.; Bhole, R.V. Effluent of Dairy Technology. *Int. Res. J. Sodh Samiksha Mulyankan* **2008**, *4*, 303–306.
11. Gonzalez, N.C.; Pérez-Sato, M.; Soni-Guillermo, E.; Valencia-Franco, E.; Carmona-Victoria, M.; Calderón-Sánchez, F. Nitric acid and hydrogen peroxide in the digestion of milk and cheeses for the detection of heavy metals. *Agro Prod.* **2022**, *IV*, 61–70. [CrossRef]
12. Danalewich, J.; Papagiannis, T.; Belyea, R.; Tumbleson, M.; Raskin, L. Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal. *Water Res.* **1998**, *32*, 3555–3568. [CrossRef]
13. Das, P.; Paul, K.K. Present Scenario of Dairy Wastewater Treatment: A State of Art Review. *Res. Sq.* **2022**. [CrossRef]
14. Ne, A. Waste Water from Industry of Milk and Dairy Products in Kragujevac. *IETI Trans. Eng. Res. Pract.* **2017**, *1*, 8–14.
15. Shete, N.P. Dairy Industry Wastewater Sources, Characteristics & Its Effects on Environment. *Int. J. Curr. Eng. Technol.* **2013**, *3*, 1611–1615. Available online: http://inpressco.com/category/ijcet (accessed on 3 May 2023).
16. Goli, A.; Shamiri, A.; Khosroyar, S.; Talaiekhozani, A.; Sanaye, R.; Azizi, K. A review on different aerobic and anaerobic treatment methods in dairy industry wastewater. *J. Environ. Treat. Tech.* **2019**, *7*, 113–141.
17. Walker, S.I. Waste water treatment in the dairy industry. *Int. J. Dairy Technol.* **2001**, *54*, 78–80. [CrossRef]
18. Bajracharya, S.; Sharma, M.; Mohanakrishna, G.; Benneton, X.D.; Strik, D.P.; Sarma, P.M.; Pant, D. An overview on emerging bioelectrochemical systems (BESs): Technology for sustainable electricity, waste remediation, resource recovery, chemical production and beyond. *Renew. Energy* **2016**, *98*, 153–170. [CrossRef]
19. Thulasinathan, B.; Jayabalan, T.; Arumugam, N.; Kulanthaisamy, M.R.; Kim, W.; Kumar, P.; Govarthanan, M.; Alagarsamy, A. Wastewater substrates in microbial fuel cell systems for carbon-neutral bioelectricity generation: An overview. *Fuel* **2022**, *317*, 123369. [CrossRef]
20. Das, S.; Das, S.; Das, I.; Ghangrekar, M. Application of bioelectrochemical systems for carbon dioxide sequestration and concomitant valuable recovery: A review. *Mater. Sci. Energy Technol.* **2019**, *2*, 687–696. [CrossRef]
21. Pandit, C.; Thapa, B.S.; Srivastava, B.; Mathuriya, A.S.; Toor, U.-A.; Pant, M.; Pandit, S.; Jadhav, D.-A. Integrating Human Waste with Microbial Fuel Cells to Elevate the Production of Bioelectricity. *BioTech* **2022**, *11*, 36. [CrossRef]
22. Koca, N. (Ed.) *Technological Approaches for Novel Applications in Dairy Processing*; IntechOpen: Rijeka, Croatia, 2018. [CrossRef]
23. Kushwaha, J.P.; Srivastava, V.C.; Mall, I.D. Sequential batch reactor for dairy wastewater treatment: Parametric optimization; kinetics and waste sludge disposal. *J. Environ. Chem. Eng.* **2013**, *1*, 1036–1043. [CrossRef]

24. Lateef, A.; Chaudhry, M.N.; Ilyas, S. Biological treatment of dairy wastewater using activated sludge. *ScienceAsia* **2013**, *39*, 179–185. [CrossRef]
25. Żyłka, R.M.; Dąbrowski, W.; Gogina, E.; Yancen, O. Trickling Filter for High Efficiency Treatment of Dairy Sewage. *J. Ecol. Eng.* **2018**, *19*, 269–275. [CrossRef] [PubMed]
26. Tocchi, C.; Federici, E.; Fidati, L.; Manzi, R.; Vincigurerra, V.; Petruccioli, M. Aerobic treatment of dairy wastewater in an industrial three-reactor plant: Effect of aeration regime on performances and on protozoan and bacterial communities. *Water Res.* **2012**, *46*, 3334–3344. [CrossRef] [PubMed]
27. Ramasamy, E.; Gajalakshmi, S.; Sanjeevi, R.; Jithesh, M.; Abbasi, S. Feasibility studies on the treatment of dairy wastewaters with upflow anaerobic sludge blanket reactors. *Bioresour. Technol.* **2004**, *93*, 209–212. [CrossRef]
28. Marassi, R.J.; López, M.B.G.; Queiroz, L.G.; Silva, D.C.V.; da Silva, F.T.; de Paiva, T.C.B.; Silva, G.C. Efficient dairy wastewater treatment and power production using graphite cylinders electrodes as a biofilter in microbial fuel cell. *Biochem. Eng. J.* **2022**, *178*, 108283. [CrossRef]
29. Das, B.; Sarkar, S.; Sarkar, A.; Bhattacharjee, S.; Bhattacharjee, C. Recovery of whey proteins and lactose from dairy waste: A step towards green waste management. *Process. Saf. Environ. Prot.* **2016**, *101*, 27–33. [CrossRef]
30. Chollangi, A.; Hossain, M. Separation of proteins and lactose from dairy wastewater. *Chem. Eng. Process. Process Intensif.* **2007**, *46*, 398–404. [CrossRef]
31. Patil, S.A.; Ahire, V.V.; Hussain, M.H. Dairy Wastewater-a Case Study. *Int. J. Res. Eng. Technol.* **2014**, *3*, 30–34.
32. Yang, P.; Zhang, R.; McGarvey, J.A.; Benemann, J.R. Biohydrogen production from cheese processing wastewater by anaerobic fermentation using mixed microbial communities. *Int. J. Hydrog. Energy* **2007**, *32*, 4761–4771. [CrossRef]
33. Hassan, A.; Nelson, B. Invited review: Anaerobic fermentation of dairy food wastewater. *J. Dairy Sci.* **2012**, *95*, 6188–6203. [CrossRef] [PubMed]
34. Fang, H.H.P.; Yu, H.Q. Acidification of Lactose in Wastewater. *J. Environ. Eng.* **2001**, *127*, 825–831. [CrossRef]
35. Suman, G.; Nupur, M.; Anuradha, S.; Pradeep, B. Assessment of toxicity in dairy waste: A Review. *IOSR J. Biotechnol. Biochem.* **2017**, *3*, 6–10.
36. Britz, T.J.; van Schalkwyk, C.; Hung, Y.T. Treatment of dairy processing wastewaters. In *Handbook of Industrial and Hazardous Wastes Treatment*; CRC Press: Boca Raton, FL, USA, 2004; pp. 673–705.
37. Report Technical Evaluation. Lactic Acid, Sodium Lactate, and Potassium Lactate. Available online: https://www.ams.usda.gov/sites/default/files/media/Lactic%20Acid%20TR%202015.pdf (accessed on 5 June 2023).
38. Zheng, Z. Extracellular Electron Transfer of S. oneidensis MR 1: Fundamentals and Applications. Technical University of Denmark. 2019. Available online: https://backend.orbit.dtu.dk/ws/portalfiles/portal/222411276/Zhiyong_Zheng.pdf (accessed on 12 June 2023).
39. Nealson, K.H.; Moser, D.P. Growth of the facultative anaerobe *Shewanella putrefaciens* by elemental sulfur reduction. *Appl. Environ. Microbiol.* **1996**, *62*, 2100–2105.
40. Sun, W.; Lin, Z.; Yu, Q.; Cheng, S.; Gao, H. Promoting Extracellular Electron Transfer of *Shewanella oneidensis* MR-1 by Optimizing the Periplasmic Cytochrome *c* Network. *Front. Microbiol.* **2021**, *12*, 727709. [CrossRef] [PubMed]
41. Edel, M.; Sturm, G.; Sturm-Richter, K.; Wagner, M.; Ducassou, J.N.; Couté, Y.; Horn, H.; Gescher, J. Extracellular riboflavin induces anaerobic biofilm formation in *Shewanella oneidensis*. *Biotechnol. Biofuels* **2021**, *14*, 130. [CrossRef]
42. Hu, Y.; Wang, Y.; Han, X.; Shan, Y.; Li, F.; Shi, L. Biofilm Biology and Engineering of *Geobacter* and *Shewanella* spp. for Energy Applications. *Front. Bioeng. Biotechnol.* **2021**, *9*, 786416. [CrossRef]
43. Chen, Y.; Wang, F. Insights on nitrate respiration by *Shewanella*. *Front. Mar. Sci.* **2015**, *1*, 80. [CrossRef]
44. Pirbadian, S.; Barchinger, S.E.; Leung, K.M.; Byun, H.S.; Jangir, Y.; Bouhenni, R.A.; Reed, S.B.; Romine, M.F.; Saffarini, D.A.; Shi, L.; et al. *Shewanella oneidensis* MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 12883–12888. [CrossRef]
45. von Canstein, H.; Ogawa, J.; Shimizu, S.; Lloyd, J.R. Secretion of Flavins by *Shewanella* Species and Their Role in Extracellular Electron Transfer. *Appl. Environ. Microbiol.* **2008**, *74*, 615–623. [CrossRef]
46. Bretschger, O.; Obraztsova, A.; Sturm, C.A.; Chang, I.S.; Gorby, Y.A.; Reed, S.B.; Culley, D.E.; Reardon, C.L.; Barua, S.; Romine, M.F.; et al. Current Production and Metal Oxide Reduction by *Shewanella oneidensis* MR-1 Wild Type and Mutants. *Appl. Environ. Microbiol.* **2007**, *73*, 7003–7012. [CrossRef] [PubMed]
47. Zang, Y.; Zhao, T.; Xie, B.; Feng, Y.; Yi, Y.; Liu, H. A bio-electrochemical sensor based on suspended *Shewanella oneidensis* MR-1 for the sensitive assessment of water biotoxicity. *Sens. Actuators B Chem.* **2021**, *341*, 130004. [CrossRef]
48. Ishii, T.; Kawaichi, S.; Nakagawa, H.; Hashimoto, K.; Nakamura, R. From chemolithoautotrophs to electrolithoautotrophs: CO2 fixation by Fe(II)-oxidizing bacteria coupled with direct uptake of electrons from solid electron sources. *Front. Microbiol.* **2015**, *6*, 994. [CrossRef]
49. Aydogdu, T.; O'mahony, J.A.; McCarthy, N.A. pH, the Fundamentals for Milk and Dairy Processing: A Review. *Dairy* **2023**, *4*, 395–409. [CrossRef]
50. Karadag, D.; Köroğlu, O.E.; Ozkaya, B.; Cakmakci, M. A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. *Process. Biochem.* **2015**, *50*, 262–271. [CrossRef]
51. Janczukowicz, W.; Zieliński, M.; Dębowski, M. Biodegradability evaluation of dairy effluents originated in selected sections of dairy production. *Bioresour. Technol.* **2008**, *99*, 4199–4205. [CrossRef] [PubMed]

52. Demirel, B.; Yenigun, O.; Onay, T.T. Anaerobic treatment of dairy wastewaters: A review. *Process. Biochem.* **2005**, *40*, 2583–2595. [CrossRef]
53. Kaur, N. Different treatment techniques of dairy wastewater. *Groundw. Sustain. Dev.* **2021**, *14*, 100640. [CrossRef]
54. Das, S.; Calay, R.K. Experimental Study of Power Generation and COD Removal Efficiency by Air Cathode Microbial Fuel Cell Using *Shewanella baltica* 20. *Energies* **2022**, *15*, 4152. [CrossRef]
55. Fazli, N.; Mutamim, N.S.A.; Jafri, N.M.A.; Ramli, N.A.M. Microbial Fuel Cell (MFC) in treating spent caustic wastewater: Varies in Hydraulic Retention Time (HRT) and Mixed Liquor Suspended Solid (MLSS). *J. Environ. Chem. Eng.* **2018**, *6*, 4339–4346. [CrossRef]
56. Santos, J.B.C.; de Barros, V.V.S.; Linares, J.J. The Hydraulic Retention Time as a Key Parameter for the Performance of a Cyclically Fed Glycerol-Based Microbial Fuel Cell from Biodiesel. *J. Electrochem. Soc.* **2017**, *164*, H3001–H3006. [CrossRef]
57. Mansoorian, H.J.; Mahvi, A.H.; Jafari, A.J.; Khanjani, N. Evaluation of dairy industry wastewater treatment and simultaneous bioelectricity generation in a catalyst-less and mediator-less membrane microbial fuel cell. *J. Saudi Chem. Soc.* **2016**, *20*, 88–100. [CrossRef]
58. Banerjee, A.; Calay, R.K.; Eregno, F.E. Role and Important Properties of a Membrane with Its Recent Advancement in a Microbial Fuel Cell. *Energies* **2022**, *15*, 444. [CrossRef]
59. Banerjee, A.; Calay, R.K.; Mustafa, M. Review on Material and Design of Anode for Microbial Fuel Cell. *Energies* **2022**, *15*, 2283. [CrossRef]
60. Boas, J.V.; Oliveira, V.B.; Simões, M.; Pinto, A.M. Review on microbial fuel cells applications, developments and costs. *J. Environ. Manag.* **2022**, *307*, 114525. [CrossRef]
61. Logan, B.E. *Microbial Fuel Cells*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2007.
62. Pallavi, C.K.; Sanjay, S.; Udayashankara, T.H. Feasibility Study on Treatability of Dairy Wastewater Employing Dual Compartment Microbial Fuel Cell. *Int. Res. J. Eng. Technol.* **2017**, *4*, 752–758. Available online: https://irjet.net/archives/V4/i7/IRJET-V4I7189.pdf (accessed on 9 May 2023).
63. Optimization, O. Electricity Generation from Synthetic Wastewater in a Laboratory Scale Microbial Fuel Cell. *Pamukkale Üniversitesi Mühendislik Bilim. Derg.* **2011**, *17*, 43–49.
64. Al-Saned, A.J.O.; Kitafa, B.A.; Badday, A.S. Microbial fuel cells (MFC) in the treatment of dairy wastewater. *IOP Conf. Ser. Mater. Sci. Eng.* **2021**, *1067*, 012073. [CrossRef]
65. Kelly, P.T.; He, Z. Understanding the application niche of microbial fuel cells in a cheese wastewater treatment process. *Bioresour. Technol.* **2014**, *157*, 154–160. [CrossRef]
66. Dai, H.N.; Nguyen, T.-A.D.; LE, L.-P.M.; Van Tran, M.; Lan, T.-H.; Wang, C.-T. Power generation of *Shewanella oneidensis* MR-1 microbial fuel cells in bamboo fermentation effluent. *Int. J. Hydrog. Energy* **2021**, *46*, 16612–16621. [CrossRef]
67. Ramirez-Nava, J.; Martínez-Castrejón, M.; García-Mesino, R.L.; López-Díaz, J.A.; Talavera-Mendoza, O.; Sarmiento-Villagrana, A.; Rojano, F.; Hernández-Flores, G. The Implications of Membranes Used as Separators in Microbial Fuel Cells. *Membranes* **2021**, *11*, 738. [CrossRef]
68. Yu, D.-Y.; Wang, G.; Xu, F.-C.; Chen, L.-M. Constitution and Optimization on the Performance of Microbial Fuel Cell Based on Sulfate-Reducing Bacteria. *Energy Procedia* **2012**, *16*, 1664–1670. [CrossRef]
69. Schneider, G.; Pásztor, D.; Szabó, P.; Kőrösi, L.; Kishan, N.S.; Raju, P.A.R.K.; Calay, R.K. Isolation and Characterisation of Electrogenic Bacteria from Mud Samples. *Microorganisms* **2023**, *11*, 781. [CrossRef] [PubMed]
70. Angelaalincy, M.J.; Krishnaraj, R.N.; Shakambari, G.; Ashokkumar, B.; Kathiresan, S.; Varalakshmi, P. Biofilm Engineering Approaches for Improving the Performance of Microbial Fuel Cells and Bioelectrochemical Systems. *Front. Energy Res.* **2018**, *6*, 63. [CrossRef]
71. Greenman, J.; Gajda, I.; You, J.; Mendis, B.A.; Obata, O.; Pasternak, G.; Ieropoulos, I. Microbial fuel cells and their electrified biofilms. *Biofilm* **2021**, *3*, 100057. [CrossRef]
72. Sonawane, J.M.; Mahadevan, R.; Pandey, A.; Greener, J. Recent progress in microbial fuel cells using substrates from diverse sources. *Heliyon* **2022**, *8*, e12353. [CrossRef] [PubMed]
73. Katagiri, H.; Imai, K. Studies on lactic acid fermentation: Observations on the mode of fermentation. *J. Agric. Chem. Soc. Jpn.* **1955**, *19*, 15–21. [CrossRef]
74. Choudhury, P.; Bhunia, B.; Bandyopadhyay, T.K.; Ray, R.N. The Overall Performance Improvement of Microbial Fuel Cells Connected in Series with Dairy Wastewater Treatment. *J. Electrochem. Sci. Technol.* **2021**, *12*, 101–111. [CrossRef]
75. Zhao, Q.; Li, R.; Ji, M.; Ren, Z.J. Organic content influences sediment microbial fuel cell performance and community structure. *Bioresour. Technol.* **2016**, *220*, 549–556. [CrossRef]
76. Zavala, M.L.; Delenne, P.R.T.; Peña, O.I.G. Improvement of Wastewater Treatment Performance and Power Generation in Microbial Fuel Cells by Enhancing Hydrolysis and Acidogenesis, and by Reducing Internal Losses. *Energies* **2018**, *11*, 2309. [CrossRef]
77. Picioreanu, C.; Katuri, K.P.; Head, I.M.; van Loosdrecht, M.C.M.; Scott, K. Mathematical model for microbial fuel cells with anodic biofilms and anaerobic digestion. *Water Sci. Technol.* **2008**, *57*, 965–971. [CrossRef]
78. Bagchi, S.; Behera, M. Methanogenesis suppression in microbial fuel cell by aluminium dosing. *Bioelectrochemistry* **2019**, *129*, 206–210. [CrossRef] [PubMed]

79. Choudhury, P.; Ray, R.N.; Bandyopadhyay, T.K.; Tiwari, O.N.; Bhunia, B. Kinetics and performance evaluation of microbial fuel cell supplied with dairy wastewater with simultaneous power generation. *Int. J. Hydrog. Energy* **2021**, *46*, 16815–16822. [CrossRef]
80. Linares, R.V.; Domínguez-Maldonado, J.; Rodríguez-Leal, E.; Patrón, G.; Castillo-Hernández, A.; Miranda, A.; Romero, D.D.; Moreno-Cervera, R.; Camara-Chale, G.; Borroto, C.G.; et al. Scale up of Microbial Fuel Cell Stack System for Residential Wastewater Treatment in Continuous Mode Operation. *Water* **2019**, *11*, 217. [CrossRef]