



Treatability and kinetic analysis of anaerobic moving bed biofilm reactor treating high strength milk permeate

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ABSTRACT

High strength milk permeate wastewater with chemical oxygen demand (COD) of 50–77 g·l⁻¹ from cheese making process was treated respectively under sub-mesophilic (20–25°C) and mesophilic (35–40°C) conditions using bench-scale anaerobic moving bed biofilm reactors (AMBBR). Organic loading rates (OLR) were gradually increased from 0.5 to 6.2 g total COD (TCOD)·l⁻¹·d⁻¹ and from 1.5 to 11 g TCOD·l⁻¹·d⁻¹, achieving maximum stable OLRs at 5.5 and 9.5 g TCOD·l⁻¹·d⁻¹ with corresponding hydraulic retention times (HRT) as 14 and 6.8 days, respectively, under sub-mesophilic and mesophilic condition. Within the maximum stable OLR levels, ratios of VFA (volatile fatty acids)/TA (total alkalinity) were well maintained under suggested limit of digester failure in most cases. Therefore, TCOD and soluble COD (SCOD) removal efficiencies were found to be 82% and 92% in sub-mesophilic treatment and 84% and 96% in mesophilic one. Kinetic analysis found the maximum substrate utilization rates (μ_{\max}) were 46.1 and 95.2 g SCOD·l⁻¹·d⁻¹ along with maximum specific methane yield (Y_{\max}) values being 0.301 and 0.313 l CH₄·g⁻¹ SCOD removed, respectively, in sub-mesophilic and mesophilic treatments.

Keywords: Anaerobic moving bed biofilm reactor (AMBBR); High strength; Milk permeate; Kinetic model; Sub-mesophilic; Mesophilic

1. Introduction

Milk permeate, a lactose rich by-product of the modern industrial production of cheese by ultrafiltration technology, has always been regarded as a low value and highly polluting mass. It retains approximately 50% of the milk nutrients and is 80–85% of the whole milk by volume. Currently, the major way to handle milk permeate is to recover lactose and salts as powder for animal feed or to produce ethanol, acetic acid and lactulose by means of bio-transformation [1–3]. However, the re-

covery technologies are not suitable to small dairy plants due to the high investment costs and low marketing profits. In this case, the transformation of milk permeate into biogas by anaerobic digestion for the reuse of energy produced in the dairy plants is preferred.

Compared with other waste treatment methods, anaerobic digestion possesses major advantages such as low cost, high energy efficiency and simple process. However, anaerobic digestion is not widespread in treating milk-related wastewaters because of poor process stability and slow reaction that requires longer hydraulic retention time (HRT). These problems might be more severe in treating effluents like milk permeate which is

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rich in components that are subject to rapid acidification because of very low bicarbonate alkalinity (usually about 2500 mg·l⁻¹ as CaCO₃), extremely high biodegradability (close to 99%) and high chemical oxygen demand (COD) concentration (up to 70 g·COD·l⁻¹) [2].

The majority of full-scale applications and research effort, until recently, has been concentrated on anaerobic digestion within the mesophilic temperature range. This has largely been due to the fact that thermophilic anaerobic digestion was too expensive (except in necessary cases such as high-temperature effluent discharges) and the belief that sub-mesophilic or psychrophilic anaerobic digestion was not viable because of low microbial activity under low-temperature conditions [4,5].

Anaerobic moving bed biofilm reactor (AMBBR) was built in this study. It employed attached-growth microorganisms grown as biofilm on the surface of plastic carriers, which was vertically moved up and down in the bulk fluid, to treat the wastewater. The main advantages of this configuration include clogging prevention, efficient mixing, and good hydrodynamics due to the vertical movement of the biofilm carriers. Process modeling is an accepted route for describing the performance of biological treatment systems and for predicting their performance [6]. However, although mesophilic digesters have been described by a variety of models, little attention has been paid to sub-mesophilic digestion.

The purpose of this study is to determine the anaerobic treatability and process stability of sub-mesophilic vs. mesophilic anaerobic digestion by AMBBR for the treatment of high strength milk permeate. Moreover, this study seeks to clarify the kinetic constants of substrate utilization and methane production in both of sub-mesophilic and mesophilic treatments by carrying out kinetic analysis.

2. Materials and methods

2.1. Wastewater characteristics

The milk permeate wastewater was collected from a local cheese factory called Fromagerie Guilloteau (Belley, France). It was stored at -20°C after collection and defrosted at 4°C about 24 h before feeding. Notable characteristics of this protein-free wastewater are the low pH and alkalinity as well as high COD, total dissolved solids and biodegradability. The features and composition of this influent are summarized in Table 1.

2.2. Inoculum

In each treatment, the anaerobic moving bed biofilm reactor (AMBBR) was inoculated with methanogenically active biomass collected from a nearby operating UASB reactor treating dairy wastewater for the past many years. The content of suspended solids (SS) and volatile suspended solids (VSS) of the inoculum were: 56.8 and

Table 1
Characteristics of milk permeate

Parameter	Value
pH	3.04–6.45
ORP (mv)	120
TCOD (g·l ⁻¹)	63.48±7.60
SCOD (g·l ⁻¹)	59.26±7.34
TSS (g·l ⁻¹)	3.80±1.37
TDS (g·l ⁻¹)	12.41±1.51
TA (g·l ⁻¹)	2.85±0.33
VFA (g·l ⁻¹)	1.46±0.52
TN (g·l ⁻¹)	0.30–0.40
TP (g·l ⁻¹)	0.35–0.45
Biodegradability	BOD/COD ? 0.95

*ORP, oxidation reduction potential; COD, chemical oxygen demand; TCOD, total COD; SCOD, soluble COD; TSS, total suspended solids; TDS, total dissolved solids; TA, total alkalinity; VFA, volatile fatty acids; TN, total nitrogen; TP, total phosphorous; BOD, bio-chemical oxygen demand.

49.4 g·l⁻¹, respectively. Other characteristics of the inoculum were: pH, 7.2; total solids (TS), 57.2 g·l⁻¹; and volatile solids (VS), 49.8 g·l⁻¹.

2.3. Biofilm carriers

Low density polyethylene Bioflow 9[®] (diameter 10 mm; height 8 mm; density 0.94; specific surface 530 m²·m⁻³) was used as biofilm carriers. One such carrier consists of small cylindrical elements with small longitudinal fins that protrude on the outside surface and an internal triangle member that divides each element into three circular sectors. The principle is the floating carriers could provide a high surface for microbial growth. Moreover, the density of carriers on which biofilm is well attached reaches 1 in normal operation. In this case, more active biomass could be retained and well mixed with wastewater inside the reactor with minimum energy required.

2.4. Reactor configuration and operation

The experiment was carried out in two identical bench-scale anaerobic reactors, respectively, under sub-mesophilic (20–25°C) and mesophilic (35–40°C) conditions. Each reactor had a working volume of 30 l (diameter 25.0 cm; height 90.0 cm; effluent outlet height 86.5 cm). The reactors were operated in periodic feeding, continuously, and controlled by programmed timers. The internal mixing was generated by a submerged pump situated at the bottom of each reactor (Fig. 1).

At the beginning of the experiment, each reactor was filled with 10 l inoculum, 18 l biofilm carriers and sufficient tap water to make up its working volume. For adaptation of the sludge to the new environment, the reac-

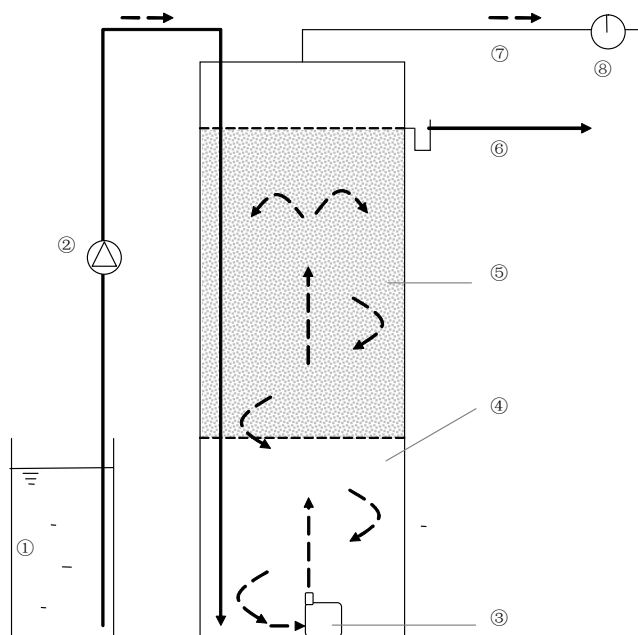


Fig. 1. Schematic diagram of bench-scale anaerobic moving bed biofilm reactor (AMBFR). 1 Influent; 2 Peristaltic pump; 3 Submerged pump; 4 Reactor; 5 Bioflow 9®; 6 Effluent; 7 Biogas; 8 Gas meter.

tors were operated for 10 days without influent prior to the continuous feeding. In the influent, nitrogen source and alkalinity buffering were provided by addition of urea and NaHCO_3 in a concentration of $0.5 \text{ g}\cdot\text{l}^{-1}$ and $2.0 \text{ g}\cdot\text{l}^{-1}$, respectively. Moreover, mineral solution Vithane® provided by Biothane Company was added into the influent by $1.0 \text{ ml}\cdot\text{l}^{-1}$. In addition, the influent pH was adjusted around 7.0 with $2.5 \text{ mol}\cdot\text{l}^{-1}$ NaOH solution.

When the continuous feeding began, milk permeate wastewater was fed into the reactors without dilution. The organic loading rates (OLR), expressed as g total COD (TCOD) per liter per day, were increased in a stepwise fashion and were implemented till the soluble COD removal yield go under 85% in each reactor. Specifically, the OLR was gradually increased from 0.5 to $6.5 \text{ g TCOD}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ in sub-mesophilic reactor and from 1.5 to $11 \text{ g TCOD}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ in mesophilic one. Therefore, the corresponding hydraulic retention times (HRT) were decreased from 116.5 to 9.7 and from 41.5 to 4.9 days.

2.5. Analytical techniques

Biogas and effluent samples were taken twice per week. Total and soluble COD values were measured by using micro method HACH (Spectrophotometer model: P/N 45600-02) and vials for COD 0–1500 ppm. pH measurements were taken with a pH meter (Model 2906, Eutech Instruments Ltd., Germany) and a pH probe (G-05992-55, Cole Parmer Instrument Co.). Volatile fatty acids (VFA), total alkalinity (TA), total solids (TS), sus-

pended solids (SS) and volatile suspended solids (VSS) were measured by following standard methods [7]. Daily biogas production was recorded using digital gas meters [8]. Methane concentration in the biogas was analyzed by Shimadzu Gas-Chromatopac GC-8A equipped with a thermal conductivity detector and recorder C-R6A. N_2 was used as the carrier gas at a flow rate of $15 \text{ ml}\cdot\text{min}^{-1}$. Column temperature was 40°C and current of detector was 90 mA.

3. Results and discussion

3.1. Reactor performance

3.1.1. Impact of high strength milk permeate on system stability

The anaerobic treatability of the milk permeate was studied in anaerobic moving bed biofilm reactors (AMBFR) respectively under sub-mesophilic and mesophilic conditions. Fig. 2 depicts the influent and effluent chemical oxygen demand (COD) concentrations as well as the corresponding organic loading rates (OLR) in each treatment. As shown in Fig. 2, high strength milk permeate with total COD (TCOD) concentrations fluctuating between $50\text{--}75 \text{ g}\cdot\text{l}^{-1}$ was directly fed into the reactors without dilution. Maximum OLRs were achieved at 6.2 and $11 \text{ g TCOD}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ with corresponding hydraulic retention times (HRT) being 9.7 and 4.9 days, respectively, in sub-mesophilic and mesophilic treatments. It is obvious that, before the maximum OLRs were applied, effluent soluble COD (SCOD_{out}) concentrations remained constant around $5 \text{ g}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ although effluent TCOD (TCOD_{out}) concentrations fluctuated in larger extents in both treatments. Specifically, in sub-mesophilic treatment, SCOD_{out} was in a range of $1.1\text{--}7.1 \text{ g}\cdot\text{l}^{-1}$ while TCOD_{out} fluctuated from 6.3 to $17.8 \text{ g}\cdot\text{l}^{-1}$. On the other hand, in mesophilic treatment, SCOD_{out} varied from 0.1 to $5.8 \text{ g}\cdot\text{l}^{-1}$ whereas TCOD_{out} varied from 4.1 to $15.7 \text{ g}\cdot\text{l}^{-1}$ (Fig. 2).

The noticeable difference between TCOD_{out} and SCOD_{out} concentrations indicates there was a considerable wash-out of sludge from the reactors. This was mainly due to the exfoliation of biofilm from the carriers, which should lead to a consequence of excessive particles contributing to the remarked high TCOD_{out} . Therefore, SCOD_{out} seems to better describe the organic degradation capacity of the reactors compared with TCOD_{out} and thus, the SCOD removal efficiency is supposed to be a better indicator of system performance.

3.1.2. Operational parameters

Fig. 3 shows the COD removal rates over the COD loading rates applied in sub-mesophilic and mesophilic reactors. In the figure, TCOD removal rate is a function of TCOD loading rate and soluble COD (SCOD) removal rate is a function of SCOD loading rate. It could be found

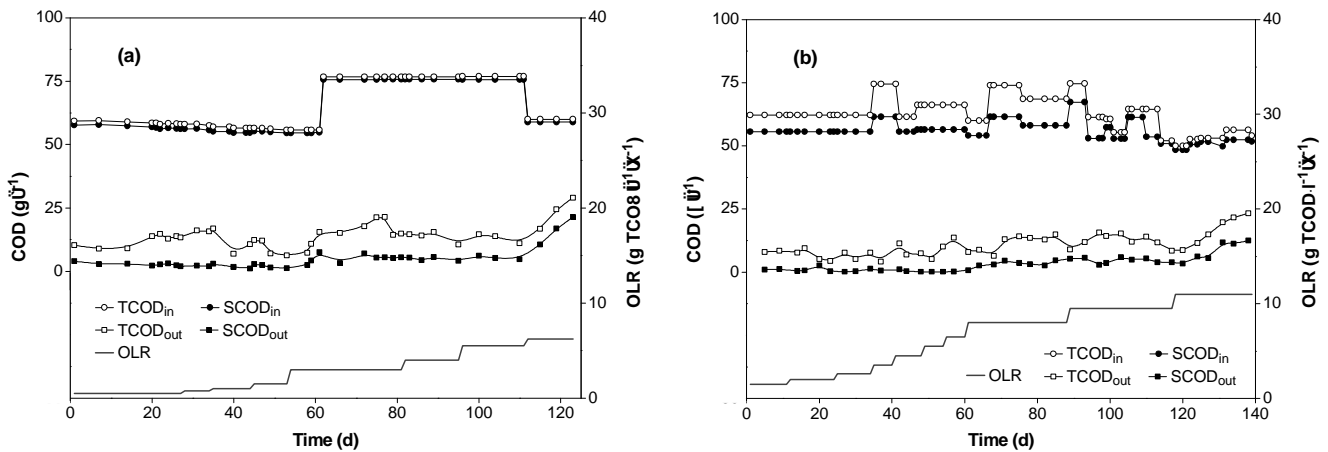


Fig. 2. Influent and effluent chemical oxygen demand (COD) concentrations as well as the corresponding organic loading rates (OLR) in (a) sub-mesophilic and (b) mesophilic treatments.

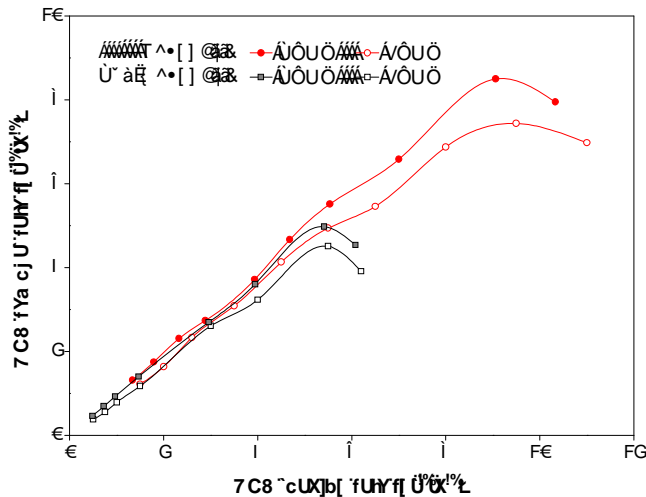


Fig. 3. COD removal rate in sub-mesophilic and mesophilic treatments. Total COD (TCOD) removal rate is a function of TCOD loading rate and soluble COD (SCOD) removal rate is a function of SCOD loading rate.

out that, compared with SCOD removal rate, TCOD removal rate goes on a slower increase with the increasing OLR in each treatment. This is even more obvious at higher OLRs applied. Another important observation from Fig. 3 is the remarked decrease of TCOD and SCOD removal rates at the maximum OLRs of 6.2 and 11 g·TCOD·l⁻¹·d⁻¹ applied in sub-mesophilic and mesophilic treatments, respectively. These sharp decreases could be explained by the accumulation of the unused volatile fatty acids (VFA) generated in the reactors and an extensive inclusion of excessive milk permeate ingredient in the effluent. For these reasons, the experiments were stopped. Thus, OLRs of 5.5 and 9.5 g·TCOD·l⁻¹·d⁻¹ were probably the maximum admissible OLRs for the stable operations of AMBBR treating undiluted high strength

milk permeate in this study, respectively, under sub-mesophilic and mesophilic conditions. Moreover, compared with sub-mesophilic condition, mesophilic condition is more favorable for the digestion of milk permeate in AMBBR in terms of the maximum stable OLR achieved.

Fig. 4 shows the TCOD and SCOD removal rates in sub-mesophilic treatment over OLR of 0.5–5.5 g·TCOD·l⁻¹·d⁻¹ and in mesophilic treatment over OLR of 1.5–9.5 g·TCOD·l⁻¹·d⁻¹. In this figure, TCOD removal rate is a function of TCOD loading rate and SCOD removal rate is a function of SCOD loading rate. The slopes in each function are the calculated COD removal efficiencies. Therefore, for sub-mesophilic AMBBR treatment of milk permeate, TCOD removal efficiency of 82% and SCOD removal efficiency of 92% could be achieved (Fig. 4a). Similarly, in mesophilic treatment, TCOD removal efficiency of 84% and SCOD removal efficiency of 95% could be achieved (Fig. 4b). As mentioned before, SCOD removal efficiency shows better information of the AMBBR performance. Thus, the comparison of the SCOD removal efficiencies obtained from both treatments (sub-mesophilic: 92%; mesophilic: 96%) shows that higher SCOD removal efficiency could be achieved in mesophilic treatment.

Regardless the high TCOD removals in either treatment, the large amount of sludge retained in the effluent that contributed to rather high TCOD_{out} concentration (Fig. 2) could not allow a direct discharge of the treated wastewater into the municipal sewage system. This problem could be easily solved by an aerobic post-treatment which would further degrade the organic matters in the AMBBR effluent. However, it should be noted that we only focused on the AMBBR treatment in this study and thus, no further aerobic treatment was carried out during the experimental period. The AMBBR effluent including both liquid and sludge was collected and transported to nearby municipal wastewater treatment plant for proper disposal.

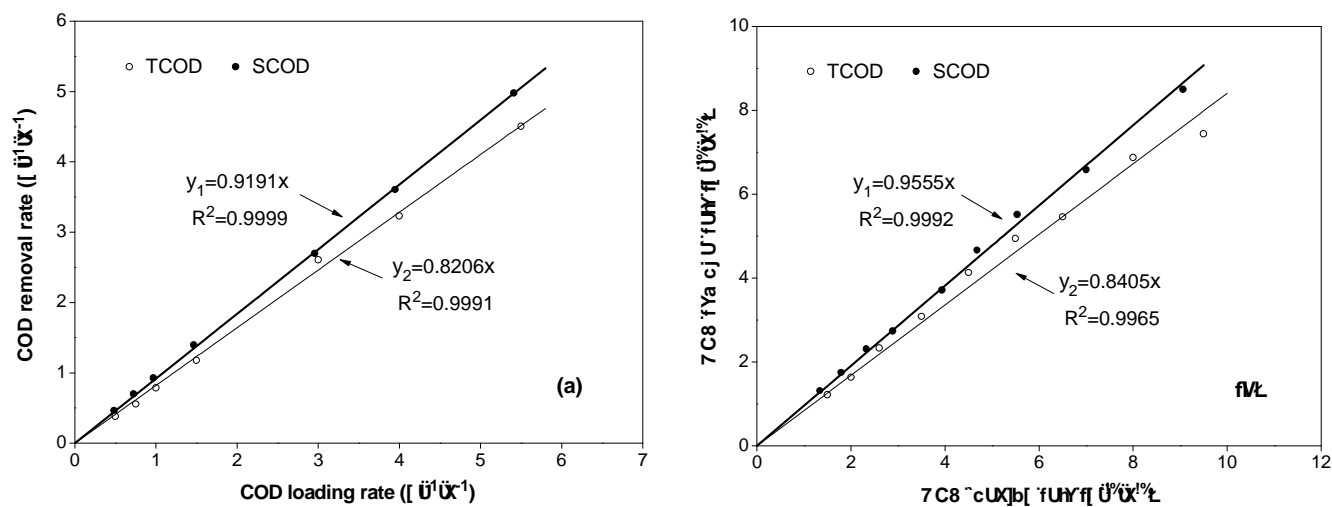


Fig. 4. COD removal rate in (a) sub-mesophilic treatment over organic loading rate of 0.5–5.5 g TCOD·l⁻¹·d⁻¹ and in (b) mesophilic treatment over organic loading rate of 1.5–9.5 g TCOD·l⁻¹·d⁻¹. TCOD removal rate is a function of TCOD loading rate and SCOD removal rate is a function of SCOD loading rate. The slopes in each function are the calculated COD removal efficiencies.

To our knowledge, no anaerobic digestion of milk permeate was reported in literatures up to date. However, Ergüder et al. [9] reported that a TCOD removal efficiency of 91–97% could be maintained in a lab-scale (0.73 l) UASB reactor treating raw cheese whey (74.5 g TCOD·l⁻¹) even at an OLR up to 24.6 g TCOD·l⁻¹·d⁻¹ under mesophilic condition. Kalyuzhnyi et al. [10] also reported that TCOD removal efficiencies higher than 90% could be achieved at OLRs up to 9.5 and 28.5 g TCOD·l⁻¹·d⁻¹ in a 3 l UASB reactor treating high strength cheese whey (up to 77 g TCOD·l⁻¹) respectively for sub-mesophilic and mesophilic regimes. Therefore, in terms of maximum stable OLR values, the aforementioned literature results are much higher than the corresponding ones achieved in this study for each temperature regime. This might be attributed to the different wastewater compositions. In this study, milk permeate was a de-proteined and nearly nitrogen-free liquid compared with cheese whey. Although urea was added into the milk permeate at a concentration of 0.5 g·l⁻¹, nitrogen source seemed insufficient for methanogenic bacteria to build up assimilative capacity of excessive VFA. This was quite notable when maximum OLRs were applied in both treatments.

Fig. 5 shows the ratio values of VFA (volatile fatty acids as equivalent acetic acid)/TA (total alkalinity as equivalent CaCO₃) in the effluent from both of sub-mesophilic and mesophilic treatments. The VFA/TA ratio can be used as a measure of process stability: when this ratio is less than 0.3–0.4 the process is considered to be operating favorably without acidification risk [11]. As is plotted in Fig. 5, most of the ratio values are lower than the suggested limit except those at OLR of 6.2 g TCOD·l⁻¹·d⁻¹

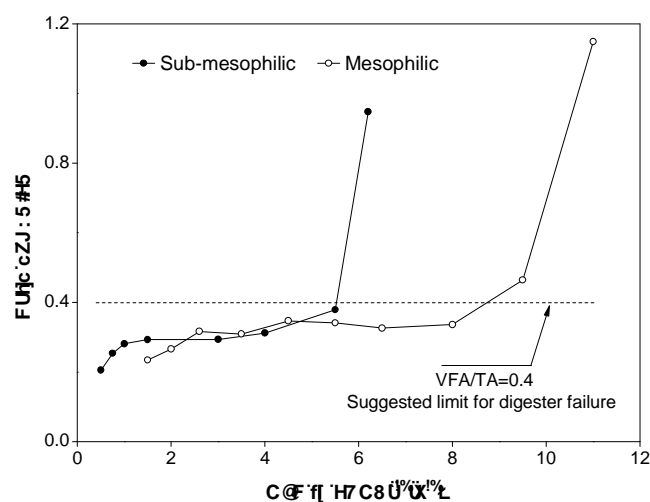


Fig. 5. Ratios of VFA (volatile fatty acids as equivalent acetic acid)/TA (total alkalinity as equivalent CaCO₃) in the effluents from both of sub-mesophilic and mesophilic treatments.

in sub-mesophilic treatment and at OLRs of 9.5 and 11 g TCOD·l⁻¹·d⁻¹ in mesophilic one. The maximum VFA/TA values recorded in both temperature regimes well mirrored the increase of SCOD_{out} as described in Fig. 2 at maximum OLRs applied. The increase of VFA/TA ratios indicates the accumulation of VFA inside the reactors. The VFA accumulation is mainly due to the fact that the lactose in milk permeate is easily degraded by acidogenic bacteria, thus causing acid inhibition to occur owing to the difference between acidogenic and methanogenic rates [12].

3.2. Kinetic analysis

3.2.1. Maximum COD utilization (Stover–Kincannon model)

The majority of mathematical models for biological systems are based on Monod kinetics [13]. Stover–Kincannon [14] working with a rotary biological contactor, assumed that the suspended biomass was negligible in comparison to the attached biomass and proposed to use the disc surface area to represent the total attached-growth active biomass concentration. However, this cannot be applied to an AMBBR system. Ahn and Forster [6] stated that suspended biomass within the interstitial void spaces of the support media is a significant factor in producing high and stable removal efficiency and thus, proposed the volume of the reactor be used instead of the surface area. Therefore, at steady state, the Stover–Kincannon model would have the form:

$$\frac{dS}{dt} = \frac{\mu_{\max}(QS_i/V)}{K_B + (QS_i/V)} \quad (1)$$

This can be linearised [6] as

$$\left(\frac{dS}{dt}\right)^{-1} = V/Q(S_i - S_e) = \frac{K_B}{\mu_{\max}} \left(\frac{V}{QS_i}\right) + \frac{1}{\mu_{\max}} \quad (2)$$

where V is the working volume (l) of reactor, Q is the flow rate ($l \cdot d^{-1}$), S_i and S_e are the substrate concentration in influent and effluent ($g \cdot l^{-1}$). Since dS/dt approaches μ_{\max} as QS_i/V , the COD loading rate, approaches infinity in Eq. (1), μ_{\max} can be deemed to be the maximum utilization rate constant. If $V/Q(S_i - S_e)$ is plotted against V/QS_i , K_B/μ_{\max} is the slope and $1/\mu_{\max}$ is the intercept point of a straight line.

Fig. 6 shows the plots for sub-mesophilic and mesophilic treatment following the linearised Stover–Kincannon model (Eq. 2). It should be noted that, $SCOD_{in}$ and $SCOD_{out}$ values were used as S_i and S_e in the calculation. The plots give values for μ_{\max} and K_B of 46.1 and 47.5 $g \text{ SCOD} \cdot l^{-1} \cdot d^{-1}$ for the sub-mesophilic treatment, as well as 95.2 and 95.5 $g \text{ SCOD} \cdot l^{-1} \cdot d^{-1}$ for the mesophilic treatment. It is obvious that the mesophilic treatment has a higher maximum utilisation rate constant (μ_{\max}) than the sub-mesophilic one. Moreover, based on the correlation constants (R^2) for both treatments in Fig. 6, it could be concluded that the modified Stover–Kincannon model is practicable in describing the performances of mesophilic as well as sub-mesophilic anaerobic moving bed biofilm reactors (AMBBR).

Another important observation from Fig. 6 is that the maximum values predicted by the modified Stover–Kincannon model are significantly higher than the maximum stable OLRs achieved in both temperature regimes, indicating that the AMBBR treatments could not achieve the μ_{\max} in this study. This might be explained by the

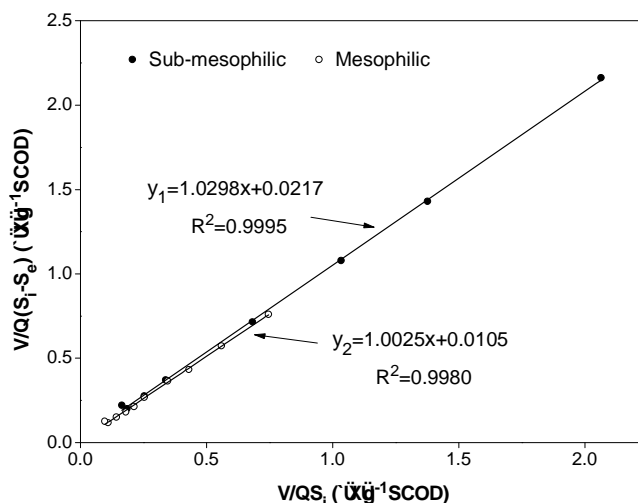


Fig. 6. Stover–Kincannon model plot for sub-mesophilic treatment over OLR of 0.5–5.5 $g \text{ TCOD} \cdot l^{-1} \cdot d^{-1}$ and for mesophilic treatment over OLR of 1.5–9.5 $g \text{ TCOD} \cdot l^{-1} \cdot d^{-1}$.

insufficient sludge retained in the reactors at the end of both treatments.

3.2.2. Maximum specific methane yield

In this study, the average specific methane yield (SMY, expressed as $l \text{ CH}_4 \cdot g^{-1} \text{ SCOD}$ removed) at each OLR applied was in a range of 0.267–0.306 $l \text{ CH}_4 \cdot g^{-1} \text{ SCOD}$ removed in sub-mesophilic treatment and 0.290–0.313 $l \text{ CH}_4 \cdot g^{-1} \text{ SCOD}$ removed in mesophilic treatment.

To derive kinetic constants for the production of methane and thus evaluate the ability of maximum specific methane yield (Y_{\max}) in both treatments, kinetic analysis was also carried out by following a similar equation of Eq. (1) proposed by Yu et al. [15]. In this equation, specific methane production ($l \text{ CH}_4 \cdot l^{-3}$ reactor volume) was supposed to be used as the biogas component. However, the more practical parameter is the specific methane yield (SMY, expressed as $l \text{ CH}_4 \cdot g^{-1} \text{ SCOD}$ removed). Therefore, the following linearised equation was tested with the current data;

$$\frac{1}{SMY} = \frac{A}{Y_{\max}} \left(\frac{V}{QS_i}\right) + \frac{1}{Y_{\max}} \quad (3)$$

where Y_{\max} is the maximum specific methane yield (SMY) and A is a constant.

Fig. 7 shows the plots for sub-mesophilic and mesophilic treatment following Eq. (3) for the determinations of methane production kinetics. Results from the Figure show that, Eq. (3) is applicable for the sub-mesophilic and mesophilic treatments when reactor was not overloaded. In spite of the remarked difference found in the μ_{\max} values (46.1 and 95.2 $g \text{ SCOD} \cdot l^{-1} \cdot d^{-1}$ respectively in sub-mesophilic and mesophilic treatments), the Y_{\max} values being 0.301 and 0.313 $l \text{ CH}_4 \cdot g^{-1} \text{ SCOD}$ removed re-

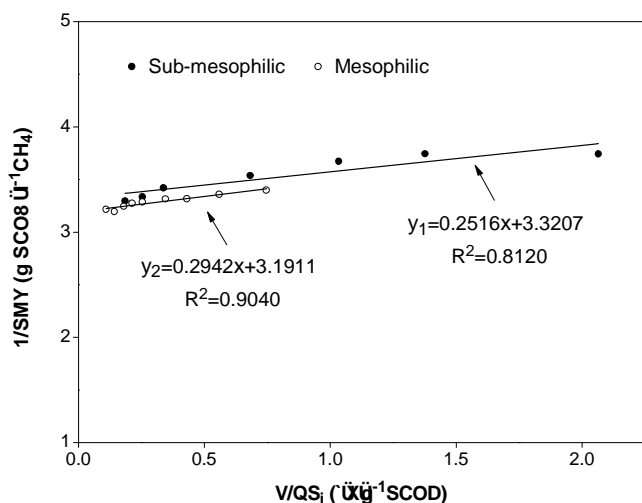


Fig. 7. Determination of the methane production kinetics for sub-mesophilic treatment over OLR of 0.5–5.5 gTCOD·l⁻¹·d⁻¹ and for mesophilic treatment over OLR of 1.5–9.5 g TCOD·l⁻¹·d⁻¹.

spectively for the sub-mesophilic and the mesophilic treatments show slight difference. This indicates that different temperature regimes did not manifest themselves in methane production capability.

4. Conclusions

This study indicated that high rate anaerobic treatments are practicable in treating high strength milk permeate using anaerobic moving bed biofilm reactors (AMBBR) under both of sub-mesophilic and mesophilic conditions. Furthermore, the following conclusions can be drawn based on the experimental results and kinetic analysis.

Maximum stable OLRs of 5.5 and 9.5 g TCOD l⁻¹·d⁻¹ with corresponding hydraulic retention times (HRT) as 14 and 6.8 days were achieved, respectively, under sub-mesophilic and mesophilic condition. Moreover, TCOD and SCOD removal efficiencies were found to be 82% and 92% in sub-mesophilic treatment and 84% and 96% in mesophilic one.

Within the maximum stable OLR level of both temperature regimes, ratios of VFA (volatile fatty acids)/TA (total alkalinity) were well maintained under suggested limit of digester failure (VFA/TA = 0.4) in most cases.

Maximum substrate utilization rates (μ_{\max}) of sub-mesophilic and mesophilic AMBBR treatments were determined as 46.1 and 95.2 g SCOD·l⁻¹·d⁻¹, proving that different temperature regimes do have notable impact on organic degradation in reactors.

Maximum specific methane yields (Y_{\max}) were estimated as 0.301 and 0.313 l CH₄·g⁻¹ SCOD removed respectively in sub-mesophilic and mesophilic treatments. These results indicate that methane production capacity of anaerobic reactors is somewhat free from temperature impact.

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References

- [1] W.B. Silveira, F.J.V. Passos, H.C. Mantovani and F.M.L. Passos, Ethanol production from cheese whey permeate by *Kluyveromyces marxianus* UFV-3: A flux analysis of oxido-reductive metabolism as a function of lactose concentration and oxygen levels, *Enzyme Microbial Technol.*, 36 (2005) 930–936.
- [2] M. Talabardon, J.-P. Schwitzgubel and P. Peringer, Anaerobic thermophilic fermentation for acetic acid production from milk permeate, *J. Biotechnol.*, 76 (2000) 83–92.
- [3] M. Villamiel, N. Corzo, M.I. Foda, F. Montes and A. Olano, Lactulose formation catalysed by alkaline-substituted sepiolites in milk permeate, *Food Chem.*, 76 (2002) 7–11.
- [4] C.Y. Lin, T. Noike, K. Sato and J. Matsumoto, Temperature characteristics of the methanogenesis process in anaerobic digestion, *Water Sci. Tech.*, 19 (1987) 299–310.
- [5] G. Lettinga, S. Rebac and G. Zeeman, Challenge of psychrophilic anaerobic wastewater treatment, *Trends Biotechnol.*, 19(9) (2001) 363–370.
- [6] J.H. Ahn and C.F. Forster, Kinetic analyses of the operation of mesophilic and thermophilic anaerobic filters treating a simulated starch wastewater, *Process Biochem.*, 36 (2000) 19–23.
- [7] American Public Health Association, Standard methods for the examination of water and wastewater, 19th ed., APHA/American Water Works Association/Water Environment Federation, Washington, DC, 1995.
- [8] R. Moletta and G. Albagnac, A gas meter for low rates of gas flow: Application to the methane fermentation, *Biotechnol. Lett.*, 4(5) (1982) 319–322.
- [9] T.H. Ergüder, U. Tezel, E. Güven and G.N. Demirer, Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors, *Waste Manag.*, 21 (2001) 643–650.
- [10] S.V. Kalyuzhnyi, E.P. Martinez and J.R. Martinez, Anaerobic treatment of high-strength cheese-whey wastewaters in laboratory and pilot UASB-reactors, *Bioresource Technol.*, 60 (1997) 59–65.
- [11] R. Borja, B. Rincon, F. Raposo, J.R. Dominguez, F. Millan and A. Martin, Mesophilic anaerobic digestion in a fluidised-bed reactor of wastewater from the production of protein isolates from chickpea flour, *Process Biochem.*, 39 (2004) 1913–1921.
- [12] E.W. Schroder and J. De Haast, Anaerobic digestion of deproteinated cheese whey in an upflow sludge-blanket reactor, *J. Dairy Res.*, 56 (1989) 129–139.
- [13] V. Saravanan and T.R. Sreerishnan, Modelling anaerobic biofilm reactors – A review, *J. Environ. Manag.*, 81 (2006) 1–18.
- [14] D.F. Kincannon and E.L. Stover, Design methodology for fixed film reaction-RBCs and biological towers, *Civil Eng. Prac. Des. Eng.*, 2 (1982) 107–124.
- [15] H.Q. Yu, F. Wilson and J.H. Tay, Kinetic analysis of an anaerobic filter treating soybean waste-water, *Water Res.*, 32 (1998) 3341–3352.