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Operation of Submerged Anaerobic Membrane Bioreactors at 20 °C: Effect of Solids Retention Time on Flux, Mixed Liquor Characteristics and Performance

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Abstract: Four flat-sheet submerged anaerobic membrane bioreactors ran for 242 days on a simulated domestic wastewater with low Chemical Oxygen Demand (COD) and high suspended solids. Organic loading was maintained around 1.0 g COD L^{-1} day^{-1}, while solids retention time (SRT) was varied from 20–90 days. This was achieved at a constant membrane flux, maintained by adjusting transmembrane pressure (TMP) in the range 1.8–9.8 kPa. Membrane fouling was assessed based on the required TMP, with mixed liquors characterised using capillary suction time, frozen image centrifugation and quantification of extracellular polymeric substances (EPS). SRT had a significant effect on these parameters: fouling was least at an SRT of 30 days and highest at 60 days, with some reduction as this extended to 90 days. Operation at SRT < 30 days showed no further benefits. Although operation at a short SRT was optimal for membrane performance it led to lower specific methane productivity, higher biomass yields and higher effluent COD. Short SRT may also have accelerated the loss of essential trace elements, leading to reduced performance under these conditions. A COD-based mass balance was conducted, including both biomass and methane dissolved in the effluent.

Keywords: anaerobic membrane bioreactors; ambient temperature; membrane fouling; mean cell residence time; wastewater treatment

Citation: Pacheco-Ruiz, S.; Heaven, S.; Banks, C.J. Operation of Submerged Anaerobic Membrane Bioreactors at 20 °C: Effect of Solids Retention Time on Flux, Mixed Liquor Characteristics and Performance. *Processes* **2021**, *9*, 1525. https://doi.org/10.3390/pr9091525

Academic Editor: Bipro R. Dhar

Received: 19 July 2021
Accepted: 24 August 2021
Published: 29 August 2021

1. Introduction

Anaerobic technologies for wastewater treatment may offer advantages over aerobic systems, as they produce methane-rich biogas and have much lower sludge yields [1,2]. Anaerobic processes are normally operated at around 35 °C, which is known to be an optimum for maintaining a high metabolic activity [3]. It is, however, rarely economical to work at this temperature when treating low-strength municipal wastewaters, as the energy yield may be lower than the parasitic energy demand for heating [4].

Although it is well-known that lower temperatures reduce the rate of biological reaction, there is increasing awareness that effective operation is possible using retained and acclimated biomass [5]. Lower temperature operation does, however, raise other issues such as the increased solubility of methane in the effluent stream; potentially lower removal efficiencies for Chemical Oxygen Demand (COD); and an increase in water viscosity that can reduce the membrane flux and change the settling characteristics of biological solids [3,6–9]. The development of the anaerobic membrane bioreactor (AnMBR) has made it possible to produce high-quality effluents while operating at ambient temperatures and at a reasonably short hydraulic retention time (HRT). A number of reviews [10–12] have demonstrated a growing interest in the application of AnMBRs to a variety of wastewater types. The successful treatment of real and simulated municipal wastewaters with biogas production in AnMBR at operating temperatures as low as 15 °C has been demonstrated [13,14].

Yet, there still remain considerable knowledge gaps and technical challenges before the technology can become more widely adopted at full scale [10,15].

The retention of biomass in an AnMBR allows mixed liquor suspended solids (MLSS) concentrations to be maintained without carry-over of solids into the effluent; it also gives the potential for selecting the MLSS concentration for optimum organic matter degradation and membrane performance [7,15]. The decoupling of solids retention time (SRT) from HRT provides a way to control the mean cell residence time (MCRT) in the reactor [8]. This approach to process control has been used extensively in aerobic wastewater treatment, as MCRT is the reciprocal of biomass growth rate and, therefore, directly influences both metabolic activity and sludge yield [16]. The simplest way to control MCRT, and, therefore, gain kinetic control over the treatment process, is through proportional biomass wastage [17]. This important kinetic control parameter has not, however, been widely used in anaerobic systems, even though MCRT has been shown to influence the production of extracellular polymeric substances (EPS) and soluble microbial products [3,18]. These are particularly important in submerged AnMBR (SAnMBR), where the membrane is directly immersed in the mixed liquor, and both EPS and soluble microbial products are known to affect membrane fouling [19,20]. In aerobic treatment systems, it is thought that a long MCRT reduces the concentration of EPS and soluble microbial products and because these are considered to be more critical in inducing membrane fouling than is the MLSS concentration, a long MCRT is usually chosen for operation [3,20,21].

Relatively few AnMBR studies to date have used MCRT or solids retention time (SRT) as a control parameter, while many have operated at very extended or near-infinite solids retention times, with the SRT in some cases determined only by the need to remove small volumes of MLSS for analysis. Studies in which SRT has been varied, however, have not always run for long enough to reach steady-state conditions: this is often defined as operation for at least 3 SRT. Without this, it is unlikely that MLSS properties will be representative in each case. Baek et al. [22] operated a 10-L AnMBR with a sidestream membrane filter on settled municipal wastewater over a range of SRT from 19–217 days but only achieved 3 SRT at the lowest value of 19 days. Yeo and Lee [23] tested SRT of 20 and 40 days in a 5-L SAnMBR operated at 23 °C on a feed of glucose at 5 g COD L^{-1}. It is likely that only 1 SRT was achieved in each case, although the duration of this experiment was not explicitly stated. Dong et al. [24,25] used the bench and pilot-scale AnMBRs with external hollow fibre membrane (HFM) units fed on municipal wastewater at 23 °C. They tested SRT of 100, 70 and 40 days, but none of these appear to have run for 3 SRT. Thanh et al. [26] reduced the SRT from 100 to 75, 50 and then 25 days over a 60-day period in a flat-sheet SAnMBR at 35 °C fed on dilute synthetic wastewater. Yurtsever et al. [27] treated high-salinity synthetic textile wastewater in aerobic and anaerobic flat-sheet MBRs at 60-days, 30-days and near-infinite SRT, but in each case for much less than 3 SRT. Ji et al. [28] altered the SRT from 53 days to near-infinite in response to changes in MLSS caused by varying load: this is a familiar alternative method for process control in aerobic systems, but they did not attempt to use SRT itself as a control parameter.

A small number of studies using SRT as the main control parameter have run for long enough to approach steady-state operation. In some cases, these have used high-strength effluents under mesophilic conditions. Dereli et al. [29,30] operated two mesophilic cross-flow AnMBR on high-strength corn stillage for 3 months at SRT of 20 and 30 days, respectively, then ran for a further 3 months at a 50-day SRT. Szabo-Corbacho et al. [31] investigated the effect of SRT in a crossflow AnMBR treating high-strength synthetic dairy wastewater at 35 °C and completed ~3 SRT at 20 and 40-day SRT. Pacheco-Ruiz et al. [32] used a low-to-intermediate strength synthetic wastewater at 36 °C and showed that a short SRT gave enhanced membrane performance but resulted in lower specific methane production and higher waste sludge yield. Very few such studies have used low-strength wastewaters at lower temperatures, however, and the limited results to date have been conflicting. When treating synthetic low-strength wastewater in a flat-sheet membrane at 25–30 °C, Huang et al. [33] noted that a longer SRT led to greater fouling when using the

same AnMBR to treat municipal wastewater, however, they also found higher fouling at a short SRT [34]. While more studies are coming through using real municipal wastewaters, at ambient temperatures, and/or in larger-scale systems [1,6,15,24,35], data interpretation and performance prediction can be challenging due to the number of influencing variables. This is especially the case where multiple factors can alter simultaneously [3,4,7], and there is thus a clear need for studies focusing on the effects of individual parameters such as SRT.

The current research aimed to assess the effect of changes in SRT on the performance of a SAnMBR treating low-strength wastewater at 20 °C, and, in particular, the influence on membrane fouling, sludge yield, COD removal efficiency, and physico-chemical properties of the MLSS once steady-state operation has been achieved. The results may thus contribute towards resolving some conflicting reports in the literature and could help to establish MCRT or SRT as a principal control parameter for anaerobic wastewater treatment systems, in much the same way as it is considered the control parameter of choice in aerobic systems.

2. Materials and Methods

2.1. SAnMBR Design and Operation

Four SAnMBRs (S1, S2, S3 and S4) were used, in which the transmembrane pressure (TMP) was applied via a gravity head [28]. Each SAnMBR had a working volume of 9.6 L, with 2.1 L of headspace (Figure 1). Each was fitted with a chlorinated polyethylene flat-plate membrane cartridge (Type 203, Kubota Corporation, Osaka, Japan) with nominal pore size 0.4 µm and effective surface area 0.113 m^2, at a membrane packing density of 0.012 m^2 L^{-1}. The SAnMBRs were fed continuously with the chilled substrate at a controlled flow achieved by maintaining a constant head differential with the outlet from the membrane cartridge lumen. The driving force for the passage of effluent through the membrane was thus gravity-induced, and the TMP could be altered within a range of 1.8–9.8 kPa by changing the differential head. The SAnMBRs could thus be operated at a constant membrane flux, allowing control of the organic loading rate to be maintained as the membranes became progressively fouled. Flow was measured on a weighing scale with 32 kg capacity and readable to 1.0 g (Model CBK 32, Adam Equipment Co Ltd., Milton Keynes, UK), with the weight of collected permeate logged automatically at 5 min intervals. The internal headspace pressure was maintained at around 0.3 kPa, with biogas released via a fermentation gas lock into a gas-impermeable collection bag.

In situ cleaning of the membrane was achieved by recirculating headspace biogas at approximately 0.57 L L^{-1} of reactor min^{-1} (corresponding to 48.5 L min^{-1} m^{-2} of membrane surface area) using a stainless-steel sparger and a diaphragm pump (AIRPO, Ningbo forever Electronic Appliances Co Ltd., Ningbo, Zheijiang, China). This gas flow also maintained the mixed liquor in suspension. SAnMBR temperature during the experimental period was maintained at 20.5 ± 0.5 °C by a stainless-steel internal heat exchange coil coupled with a thermocirculator (FC15 and FH15V, Grant Instruments Europe BV, Amsterdam, Netherlands). Feed and SAnMBR temperature were continuously recorded by solid-state IC temperature probes (LM35DZ, Texas Instruments, Dallas, TX, USA) and a datalogger (Model U3-LV, Labjack Corporation, Lakewood, CO, USA).

2.2. Inoculum and Substrate

The substrate used was a synthetic wastewater [36], which was prepared fresh each day as a concentrate and diluted to the required working strength. It was designed to give a C:N:P ratio of around 100:20:4 and solids contents similar to those of typical municipal wastewaters [37,38].

The four AnMBRs were seeded from a mesophilic anaerobic digester at a municipal wastewater treatment plant in Southampton, UK, and the headspace was purged with nitrogen. To allow temperature acclimatisation they were initially fed with the concentrated substrate at an organic loading rate (OLR) of 0.5 g COD L^{-1} day^{-1} for 48 days (not counted as part of the trial). A total of 50% of the MLSS was then removed and replaced by tap water to reduce the solids content. From this point onwards (taken as day 0 of the experimental

period) the SAnMBRs were fed with substrate prepared by diluting the concentrate to the required COD strength. The SAnMBRs were then run at different SRTs in 3 experimental phases (EP), giving a total operational period of 242 days. Details of the applied SRT, TMP and other operational parameters are given in Table A1.

Two trace element stock solutions were used containing (g L^{-1}): Al 0.1, B 0.1, Co 1.0, Cu 0.1, Fe 10.0, Mn 1.0, Ni 1.0, Zn 1.0; and Se 0.1, Mo 0.1, W 0.1.

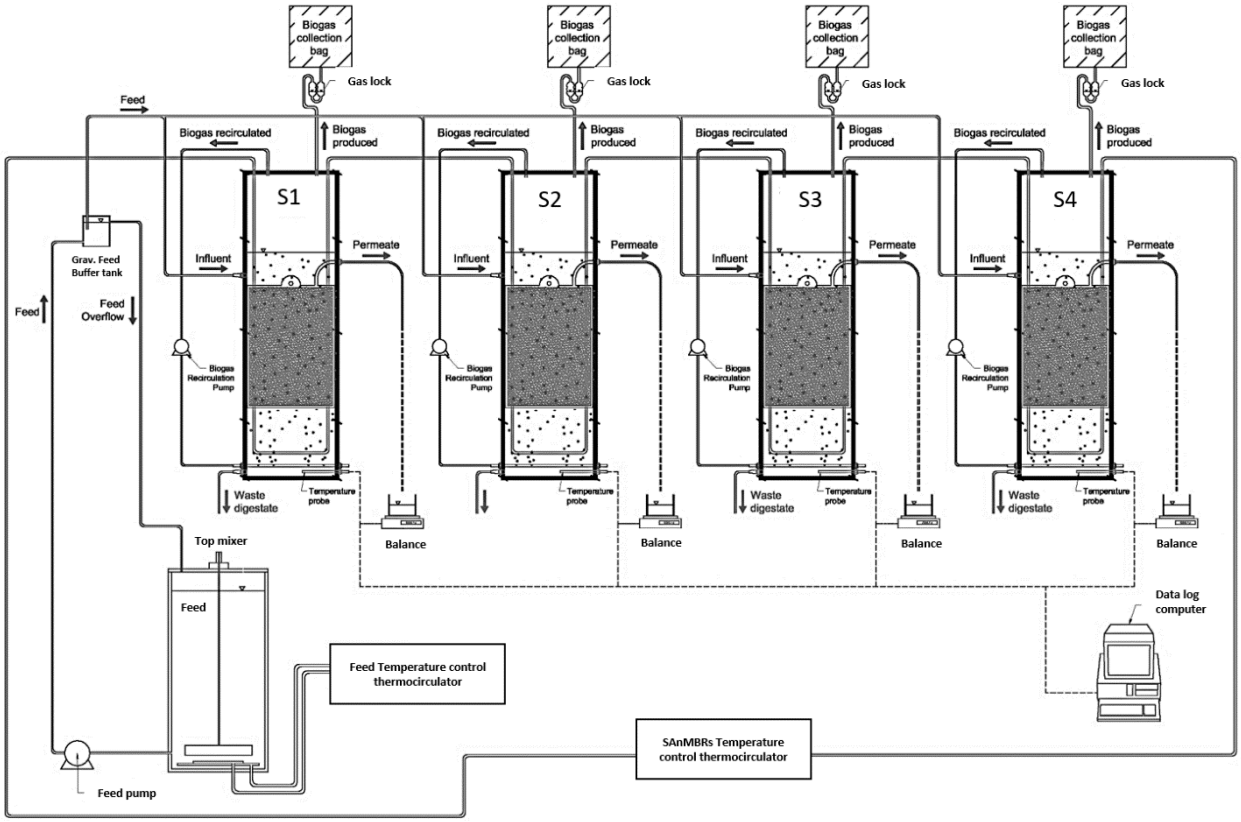

Figure 1. Schematic of experimental set-up for SAnMBRs.

2.3. Performance and Stability

Membrane performance was evaluated on the basis of the flux achieved at constant TMP, calculated as described in Pacheco-Ruiz et al. [36]. SAnMBR performance and operational stability were assessed on the basis of COD percentage conversion, specific methane production (SMP) per g of COD removed, MLSS and mixed liquor volatile suspended solids (MLVSS) concentrations, mixed liquor pH, EPS content and composition, capillary suction time (CST) (Triton Electronics Ltd., Dunmow, Cambridge, UK), and frozen image centrifugation (FIC) (Triton Electronics, UK). FIC uses a technique in which a 'frozen image' of the sample is generated by matching the frequency of a stroboscopic light to the centrifuge rotor speed, allowing measurement of the height of the solid-liquid interface in real time without interrupting the test. The centrifugation speed was fixed at 660 ± 10 rpm with observations made every minute up to 8 min. COD of fresh feed, feed after chilled storage for 24 h, and AnMBR effluent was measured using a closed-tube reflux method with titrometric end-point determination [39]. Biogas composition was analysed by gas chromatography (GP-3400, Varian Inc., Palo Alto, CA, USA) using 36% CO_2 with 64% CH_4 (v/v) (BOC, Guildford, Surrey, UK) as a standard gas. Biogas volumes were determined by a weight-type gasometer [40] and are reported at standard temperature and pressure (STP, 0 °C and 101.3 kPa).

Organic loading rate (OLR) and SMP were calculated using the average of the COD values for the fresh and stored feed. The reported SMP value includes both methane in gas

collected from the reactor headspace and dissolved methane in the effluent. This allows comparison of SAnMBR performance under different sconditions since the proportion of methane that leaves the system in the effluent will differ at different flux rates. Dissolved methane content was estimated based on Henry's Law using 20 °C saturation concentration. The resulting average value 29.0 mL CH_4 L^{-1} was confirmed by empirical measurement, using the method in Walsh and McLaughlan [41].

MLSS and MLVSS concentrations were quantified using Standard Method 2540-D [42]. pH measurements were made using a pH meter (3310, Jenway Ltd., London, UK) calibrated in pH 4, 7 and 9.2 buffers (Fisher Scientific UK Ltd., Loughborough, Leicestershire, UK). For the COD mass balance, the only input was influent COD while outputs were taken as effluent COD, COD in gaseous or dissolved methane and COD in biomass. The last of these was estimated from daily MLSS removal (g MLVSS day^{-1}) divided by the average ratio of COD/VSS in the mixed liquor (g COD g^{-1} MLVSS). COD balances did not consider changes in stored biomass, as small errors in MLSS and MLVSS measurement could introduce large variations in the overall result. Thus the COD balances were only valid for steady-state conditions or other periods with stable solids contents. The COD value of methane was taken as 2.855 g COD L^{-1} CH_4 based on stoichiometric considerations.

EPS was extracted using the formaldehyde plus NaOH procedure [43], modified in accordance with Domínguez et al. [44] and Liang et al. [45] to enable identification of bound and soluble components. Soluble EPS was extracted by centrifugation of mixed liquor, as suggested by Chabaliná et al. [46]. EPS composition was quantified by measuring the concentration of carbohydrate and protein using colorimetric methods. Carbohydrate was determined by the phenol-sulphuric acid method [47], using a glucose standard. Protein contents were analysed according to the modified Lowry Folin–Ciocalteu method suggested by Frølund et al. [48], with bovine serum albumin as a standard.

3. Results and Discussion

3.1. Operational Performance

Figure 2 presents graphical data on the operation and performance of the four AnMBRs during the 242-day experimental period, with results summarised in Tables A1 and A2. Discussion of key parameters in relation to operating conditions during each phase of the experimental period is provided below.

3.1.1. Membrane Flux, TMP, MLSS, HRT and OLR

Start-up (days 0–59). After the 48-day temperature acclimatisation phase and following dilution, the MLSS in each SAnMBR was initially 16.2 g L^{-1}. No sludge was wasted for the next 10 days, after which time proportional wasting was introduced with the aim of applying a SRT of 90 days (Figure 2a). In the following period, a number of changes were made to the TMP and feed concentration in order to find a combination of conditions that allowed acclimatisation to an increase in OLR. The TMP was initially set at 7 kPa, giving an initial flux of 16.1 L m^{-2} h^{-1}, which after 5 days had decreased to 10.2–11.0 L m^{-2} h^{-1} (Figure 2b). This high flux produced short HRTs with low COD removal rates of between 54–64%, and on day 9 TMP was reduced from 7.0 to 2.5 kPa with the aim of reducing the flux (Figure 2c). The flux remained high, however, at around 9.9–10.6 L m^{-2} h^{-1}. Thus to avoid organic overloading, the COD of the feed was reduced to around 160 mg L^{-1}. The pH immediately rose to 6.9–7.0, and the COD removal rate increased to 74–80%. The OLR was subsequently increased to 0.9, 1.0, 1.1 and 1.5 g COD L^{-1} day^{-1} stepwise at 3-day intervals by increasing the feed concentration. This led to changes in gas composition, with a higher methane content indicating greater biogas production, but the last increment in OLR resulted in a fall in pH to 6.6 and a decline in %COD removal. The OLR was, therefore, reduced on day 22 to 0.75 g COD L^{-1} day^{-1} by reducing the feed concentration, whilst the flux was maintained at 8.0–8.8 L m^{-2} h^{-1}. To improve COD removal, on day 28, the flux was then further reduced to around 6 L m^{-2} h^{-1} by reducing the TMP to 2.2 kPa (Figure 2c), and from this time onward, SAnMBR performance improved to a

point where between day 40 and 52 the OLR was successfully raised to 1.0 g COD L^{-1} day^{-1}. By day 60, stability had been achieved in all four SAnMBRs, with flux rates between 6.3–6.7 L m^{-2} h^{-1}, and MLSS contents from 12.4–12.7 g L^{-1} (Figure 2d), COD removal rates between 88–93% (Figure 2e), and stable biogas composition and production. At this point, the start-up phase was considered complete.

Experimental phase 1 (EP1, days 60–111). On day 60, the SRT was reduced to 30, 45 and 60 days in S1, S2 and S3, respectively, by increasing the volume of mixed liquor removed each day, while the SRT in S4 remained unchanged at 90 days. At the start of this phase, the TMP in all four SAnMBR was 2.5 kPa, while flux rates were 6.7, 6.4, 6.3 and 6.4 L m^{-2} h^{-1} in S1, S2, S4 and S4, respectively (Figure 2b,c). As the flux gradually declined, TMP was adjusted in each SAnMBR individually, with the aim of stabilising at a value of around 5 L m^{-2} h^{-1}. Feed concentrations were also modified slightly with respect to the achieved flux, with the aim of providing a consistent OLR of around 1 g COD L^{-1} day^{-1}. The amount of adjustment applied during this phase was, however, much less than that required in the start-up period.

By day 67, the effect of the applied changes in SRT was already evident in S3, where the onset of membrane fouling was indicated by the need for substantial increases in TMP to maintain a flux of 5 L m^{-2} h^{-1}. Similar behaviour soon followed in S2 and S4, which from day 80 also needed higher TMPs to achieve the target flux value, while in S1 it became necessary to reduce the TMP as the flux was increasing. At this stage, it was concluded that a flux of 5 L m^{-2} h^{-1} was too high for these experimental conditions. On day 100, the target was, therefore, reduced to 4 L m^{-2} h^{-1}, achieved by applying reduced TMPs of 1.8, 2.9, 4.3 and 2.2 kPa in S1, S2, S3 and S4, respectively (Figure 2c). This gave HRTs of approximately 20 h and a OLR of around 1.0 g COD L^{-1} day^{-1}, at an average COD concentration in the feed of 860 $\pm$ 23 mg COD L^{-1}. Minor variations in feed concentration here were caused by slight day-to-day variations in batch preparation and in analytical results, with no further deliberate adjustments being made to influent strength. In S1, S2 and S4 a flux of 4 L m^{-2} h^{-1} was maintained at constant TMP until the end of EP-1. In contrast, the flux in S3 continued falling irrespective of increases in the TMP, which at the end of this phase had reached 6.3 kPa (Figure 2c), giving a final OLR of 0.9 g COD L^{-1} day^{-1} at an HRT of 22.5 h in this reactor. The changes in SRT were also reflected in MLSS concentrations, with values between 12.4–12.7 g L^{-1} in all SAnMBR at the start of the phase diverging to 6.2, 7.3, 8.7 and 11.7 g L^{-1} in S1, S2, S3 and S4, respectively (Figure 2d).

Experimental phase 2 (EP-2, days 112–160). In response to the changes observed in EP-1, at the beginning of EP-2 the SRT in S3 was reduced in one step from 60 to 30 days, at an initial TMP of 6.3 kPa. S1, S2 and S4 remained at their previous SRT of 30, 45 and 90 days with corresponding initial TMPs of 1.7, 2.9 and 2.2. While flux in S1 and S2 remained steady at 4 L m^{-2} h^{-1} without any adjustment of TMP, flux in S3 continued falling, despite continuing rises in TMP (Figure 2c). On day 143, the TMP in S3 reached the limiting value of 9.8 kPa, at a flux rate of 3.6 L m^{-2} h^{-1}. The flux in S4 remained steady until day 115 when it began to decline, necessitating increases in the TMP. By day 160, S4 had also reached the maximum TMP at a flux of 3.2 L m^{-2} h^{-1} (Figure 2b). In S1 and S2, the constant flux rates resulted in stable HRT of around 20.5 h and an OLR of 1.0 g COD L^{-1} day^{-1} for both SAnMBRs. In contrast the continuous decline in flux in S3 and S4 led to higher HRTs of 28.5 and 26.6 h with OLR of 0.7 and 0.8 g COD L^{-1} day^{-1}, respectively, at the end of EP-2.

MLSS concentrations in this phase continued to reflect the different SRT, with values in S1 and S2 stabilising at around 4.4 and 5.8 g L^{-1}, respectively (Figure 2d). The fall in MLSS in S3 reflected the change in SRT to 30 days at the start of the phase, and the reduction in applied OLR due to lower flux, which resulted in a MLSS concentration of 4.3 g L^{-1} in S3 at the end of EP-2. MLSS concentrations in S4 initially stabilised at 10.8 g L^{-1} (Figure 2d) but by the end of the phase had fallen to 9.5 g L^{-1} as a result of the decrease in flux rate and consequently in OLR.

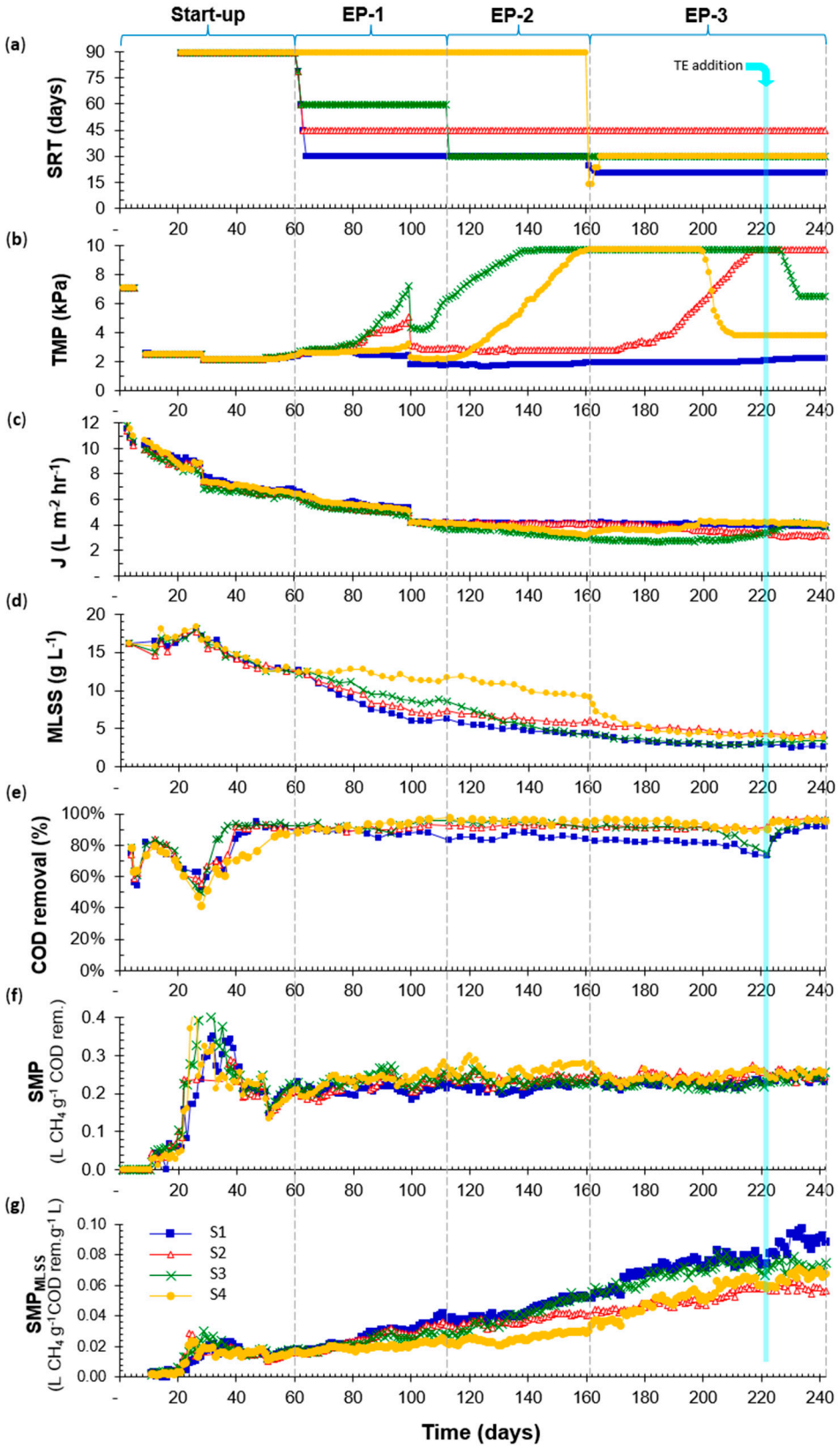

Figure 2. Operational parameters in SAnMBRs during the whole experimental period: (**a**) SRT; (**b**) TMP; (**c**) flux (daily average); (**d**) MLSS; (**e**) COD removal; (**f**) SMP; (**g**) specific SMP (MLSS basis). Vertical dotted lines indicate start of experimental phases.

At the end of EP-2, S1 had completed 101 days (equivalent to 3.4 solid retention times) at a 30-day SRT and was, therefore, regarded as having achieved steady-state operation in these conditions.

Experimental phase 3 (EP-3, days 161–242). At the start of this phase, SRT was reduced to 20 days in S1, maintained at 45 days in S2 and at 30 days in S3, and reduced to 30 days in S4. At this point, S4 had completed only 160 days at a 90-day SRT, equivalent to 1.78 solid retention times or approximately 83% washout of the MLSS originally present at the start of EP-1, but it was clear that operation was no longer sustainable within the limits of the target flux and TMP. As an alternative to the slow reduction in MLSS content occurring in S3, however, MLSS in S4 was reduced abruptly from 9.1 to 7.2 mg L^{-1}, targeting a value close to that expected for a 30-day SRT.

In the case of S4, this reduction was achieved by removing a known volume of mixed liquor and replacing it with influent, to maintain a constant working volume in the SAnMBR. The process was repeated on four consecutive days, until the MLSS concentration approached the target value. The daily volume of mixed liquor replaced was 700 mL on days 160 and 161, and 400 mL on days 162 and 163.

In S1 at a 20-day SRT, the flux during this phase was successfully maintained at 4 L m^{-2} h^{-1}, with only a slight TMP increase to 2.2 kPa. In contrast, flux in S2 at a 45-day SRT remained steady at 4 L m^{-2} h^{-1} until day 180 when it began to fall, necessitating repeated increases in TMP (Figure 2c). By day 216, TMP in S2 had reached the limiting value of 9.8 kPa, and from then on flux continued gradually to decline, stabilising at around 3.2 L m^{-2} h^{-1} by the end of EP-3. Flux in S3 at a 30-day SRT continued to fall (Figure 2b), reaching its lowest value of 2.7 L m^{-2} h^{-1} on day 183. After this it slowly recovered, up to the 4 L m^{-2} h^{-1} target by day 228 (Figure 2b). Over the next 5 days, TMP in S3 was reduced to 6.5 kPa, while flux remained steady at 4.1 L m^{-2} h^{-1} to the end of the experiment.

The sharp reduction in MLSS in S4 produced an immediate increase in flux, reaching 4 L m^{-2} h^{-1} by day 200 (Figure 2b). Over the next 10 days, the TMP was reduced to 3.8 kPa, while a steady flux of 4.1 L m^{-2} h^{-1} was maintained until the end of EP-3.

In S1 at a 20-day SRT, the HRT and OLR remained steady at around 21.0 h and 1.0 g COD L^{-1} day^{-1}. Similar values were also achieved in S3 and S4 at a 30-day SRT once they approached the same sustainable rate of 4 L m^{-2} h^{-1}. In S2 at a 45-day SRT a continuing slow decline in flux increased the HRT to 26.3 h with a corresponding fall in OLR to 0.8 g COD L^{-1} day^{-1} by the end of the phase.

At the end of EP-3, reactors S1, S2 and S3 had, respectively, completed 82, 183 and 131 days at 20-, 45- and 30-day SRT (equivalent to 4.1, 4.1 and 4.4 solid retention times in each case), making the results representative of steady-state operation in these conditions. S4 had completed 82 days at a 30-day SRT, corresponding to 2.73 solid retention times with removal of around 93.5% of the MLSS present at the start of EP-3. It was, therefore, regarded as approaching steady-state operation.

The above results indicate the complexity of interactions between SRT, required TMP, flux, MLSS, HRT and OLR, and factors associated with this are discussed in the following sections.

3.1.2. COD Removal Rates and TE Requirements

During the start-up period COD removal initially showed quite a high variability, which stabilised towards the end of the period. COD removal rates were similar in all four SAnMBRs for the first 20 days of EP-1 at 89–92% (Figure 2e). Differences in SRT began to affect COD removal during EP-1, however, with S1, S2, S3 and S4 at 30-, 45-, 60- and 90-day SRTs reaching the end of the phase with removal rates of 88%, 92%, 96% and 98%, respectively. In EP-2, COD removal rates in S1, S2 and S4 stabilised at 85 ± 2%, 93 ± 1% and 96 ± 1%, respectively. In contrast, the removal rate for S3, in which the SRT had been reduced from 60 to 30 days, fell steadily from 95% to 91%.

In the first 40 days of EP-3, there was a slight decrease in COD removal in all four SAnMBRs (Figure 2e), followed by significant falls of 8% and 20% in S1 and S3, respectively,

between days 204 and 222. It was hypothesised that this was due to a lack of trace elements (TE), since the shorter SRT in these reactors meant they had the highest overall biomass turnover, and thus the greatest risk of depletion of essential micro-nutrients. Based on this, for a 3-day period from day 223, trace elements were added to the feed for all four SAnMBRS at a dosage of 0.1 mL of each TE solution per litre of dilute influent. This led to an immediate rise in COD removal in all SAnMBRs, which stabilised at 92%, 97%, 95% and 96% in S1, S2, S3 and S4, respectively, by the end of EP-3. It was, therefore, likely that some of the decline in COD removal during this period was due to TE deficiencies, but removal rates after the TE supplementation were still slightly lower at the shorter SRTs. Other authors [30,31] have also reported slightly higher COD removal rates at longer SRT, though these studies involved high-strength substrates. In contrast, Huang et al. [33] observed no significant differences in COD removal rates for low-strength synthetic wastewater at 30-day, 60-day and near-infinite SRT.

Determination of TE requirements in AnMBR is likely to be especially challenging due to the combined effects of uncoupled liquid and solid retention times with uncertainties on bioavailability and partitioning. A number of studies have reported the addition of trace elements to feed without providing detailed justifications of the concentration or dosages used [14,19,23,33,49]. Yu et al. [50] investigated the effects of individual and combined TE supplementation on samples from the methanogenic reactor in a 2-phase AnMBR treating industrial starch wastewater at 37 °C.

They reported little effect from low doses, but with an SRT of 200 days and inoculum from a municipal sewage treatment plant, it is possible that washout of some TE to critical levels had not yet occurred. Sierra et al. [51] looked at the partitioning of trace elements B, Co, Cu, Mg, Mn, Mo, Ni, Se and W and at the effect of additional supplementation with Co and W in an AnMBR treating a highly saline phenolic wastewater. Additional Co had little effect, but W was beneficial. Doubling the overall TE dose also improved performance, and they concluded that bioavailability and partitioning were affected by high salinity. Thanh et al. [26] examined the effects of HRT, SRT and pH on bioavailability and speciation of Co, Fe, Mn, Mo, Ni and Zn. When reducing the SRT from 100 to 75, 50 and then 25 days over a 60-day period, they found varying depletion rates for different metals depending on affinity and previous accumulation. While the total trace metal content fell with the reduction in MLSS, this was partly countered by a change in speciation towards more bioavailable forms for all metals except Mn and Ni. While these studies have shown the importance of TE in ANMBR, relationships between TE, SRT and other operating parameters and optimum dosing strategies for these systems are likely to be key areas for future work [51,52].

3.1.3. COD Balances and Dissolved Methane

COD balances (shown in Figure 3) indicated that a substantial fraction of the COD was converted to methane, including a considerable proportion dissolved in the effluent. The response to the addition of TE on day 223 can also be clearly seen. COD balances for steady-state periods closed at between 92–96% in this work.

Small errors in COD balances are typical, and studies reporting better closure tend to operate with higher strength substrates and/or temperatures or at a larger scale [31]. Closures of 93–94% were obtained for a 20-L SAnMBR treating municipal wastewater at 25 °C at HRT from 4–12 h [28], while a slight excess of around 101% was reported for a 5 m^3 SAnMBR operating at the same temperature on a similar municipal effluent [1]. The missing fraction in the COD balance could be attributable in part to small quantities of biogas leaving the SAnMBRs through the permeate line in gaseous form as bubbles [36] and by any H_2S fraction in the biogas. H_2S was not taken into account in the COD balance as concentrations in the biogas were considered to be low, but the inclusion of this component may be essential in the treatment of protein- or sulphate-rich wastewaters [53]. Using Henry's Law to estimate the amount of CH_4 in the effluent may also lead to underestimation, as the calculation assumed a saturation concentration, while the elevated

TMP could cause additional dissolution and apparent methane supersaturation in the effluent [8]. In such cases, the actual volume of dissolved methane lost would be greater than the estimated value, especially at lower operating temperatures where saturation concentrations are higher. Differences between observed and simulated mass flows of methane in pilot and bench-scale sidestream AnMBRs treating screened municipal wastewater at ambient temperature were attributed to oversaturation [24], but were insufficient to account for errors of ~20% in COD balances, which may have been linked to the absence of steady-state conditions as well as to the reduction of sulphates and ferric iron. Methane oversaturation was also reported in a pilot-scale gas-sparged sidestream AnMBR operating on screened municipal wastewater at temperatures of 18.8–31.5 °C [12]. Yeo and Lee [23] noted variations in dissolved methane content with SRT in SAnMBR at 23 °C fed on glucose at 5.2 g COD L^{-1} with an HRT of 10 days. They reported oversaturation of methane and a lower biogas methane percentage at a 20-day SRT and presented COD-based balances closing at 3% and 7% for the 20 and 40-day SRT, respectively.

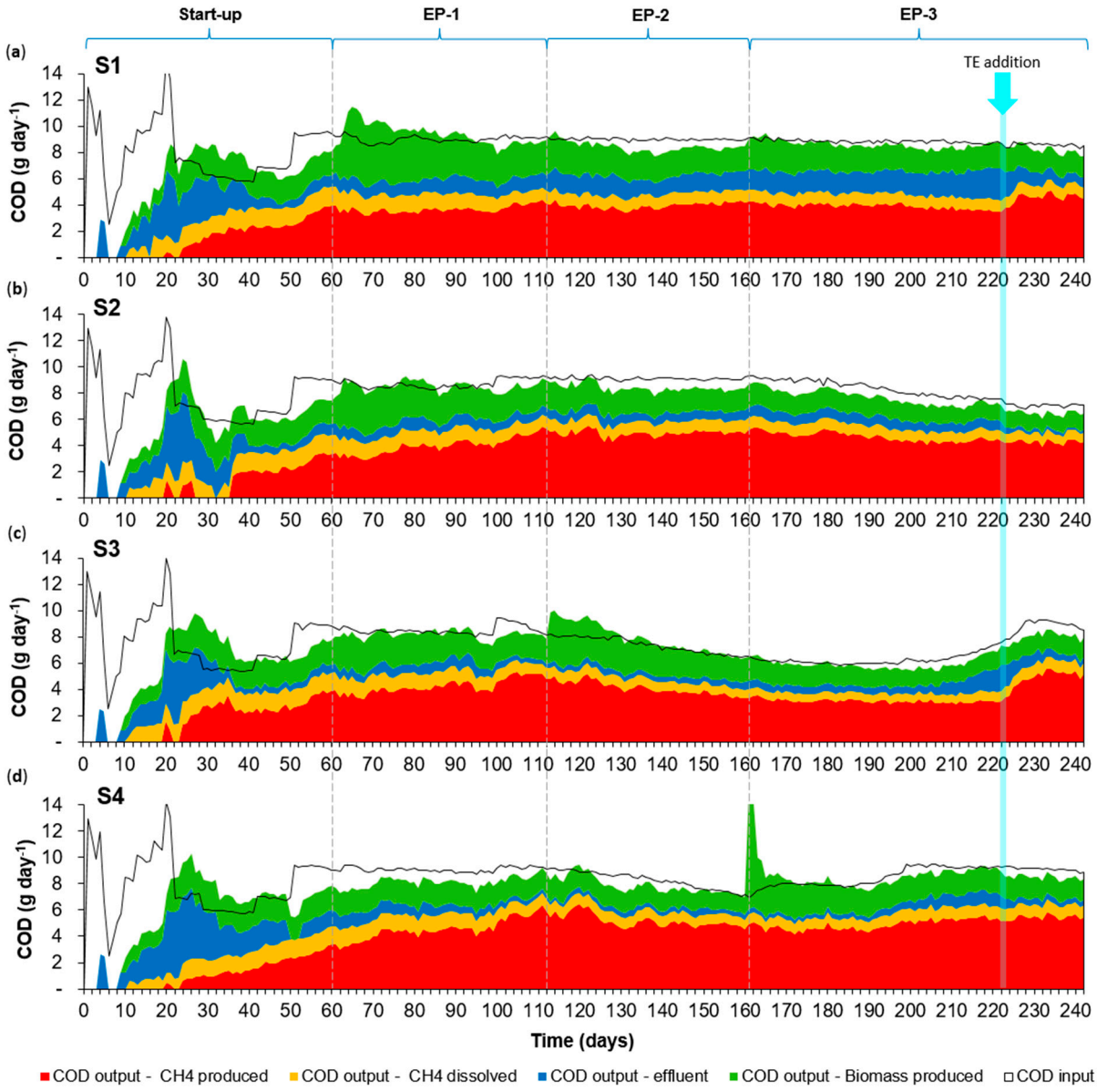

Figure 3. COD balances in SAnMBRs during the whole experimental period: (**a**) S1, (**b**) S2, (**c**) S3 and (**d**) S4. Vertical dotted lines indicate the start of experimental phases. Values for control parameters (SRT and TMP) in each phase are shown in Figure 2 and Table A2.

3.1.4. Specific Methane Productivity

Biogas composition for all SAnMBRs remained stable throughout the three experimental phases at 78% CH_4, 8% CO_2 and 14% nitrogen, the latter from headspace equilibration with atmospheric gases dissolved in the influent. SMP in all SAnMBRs showed the same trend during start-up, beginning at zero, increasing sharply to between 0.29–0.42 L CH_4 g^{-1} COD as residual feed and intermediate products were consumed, then stabilising at 0.20–0.22 L CH_4 g^{-1} COD. As can be seen from Figure 2f and Table A1, during the stable operating periods of each phase, the SMP in L CH_4 g^{-1} COD removed (COD_{rem}) was around 0.23 ± 0.01 for operation at a 20-day SRT in S1; 0.22 ± 0.01, 0.23 ± 0.01 and 0.24 ± 0.01 at a 30-day SRT in S1, S3 and S4, respectively; 0.25 ± 0.01 at a 45-day SRT in S2; and 0.26 ± 0.02 at a 90-day SRT in S4. SMP at a 60-day SRT in S3 was around 0.23 ± 0.02 L CH_4 g^{-1} COD_{rem}, although full steady-state operation under these conditions was not achieved. These results again demonstrate a decline in SMP at the shorter SRT, most likely due to the incorporation of a greater proportion of carbon into microbial biomass at higher growth rates.

This outcome was consistent with the results of earlier research at 36 °C [32], with the lower SMP values most probably due to reduced rates of reaction at a lower operating temperature. Huang et al. [33,34] also found that SMP rose with increases in SRT in the treatment of low-strength wastewaters. They reported values of 0.13, 0.20 and 0.22 L CH_4 g^{-1} COD_{rem} at SRT of 30, 60 and infinite days respectively in treatment of synthetic wastewater at OLR from 1.1–1.67 g COD L^{-1} day^{-1} [33]; and around 0.03, 0.08 and 0.08 L CH_4 g^{-1} COD_{rem} at SRT of 30, 60 and 90 days, respectively, when treating municipal wastewater at an OLR of around 1 g COD L^{-1} day^{-1} [34]. Their suggested explanation was that the longer SRT would provide better conditions for methanogenesis, allowing higher biogas productivity. No reason was given, however, for the very low values of SMP per g of COD removed when treating real municipal wastewater [34]. SMP values obtained at 20 °C in the current work are higher than those found by Huang et al. [33,34] at 25–30 °C. This may be partly due to the loss of dissolved methane in the effluent, which was not considered in their first study [33]. The pattern of lower SMP values at shorter SRTs is nevertheless evident in both of these studies and in this and earlier work at 36 °C [32]. Dong et al. [24] also reported a decline in SMP from 0.13 to 0.10 and then 0.08 L CH_4 g^{-1} COD_{rem} as SRT was stepped down from 100 to 70 and then 40 days over a 400-day experimental period when treating municipal wastewater at 23 °C.

Consideration of the SMP normalised to MLSS (SMP_{MLSS}) indicated that the biomass methane conversion efficiency was higher at shorter SRT (Figure 2g), as also seen in prior work at 36 °C [32]. SMP values obtained were similar to those at 36 °C, with a maximum of 0.095 L CH_4 g^{-1} COD_{rem} L g^{-1} MLSS for operation at a 20-day SRT in S1; and around 0.2 L CH_4 g^{-1} COD_{rem} L g^{-1} MLSS during a stable performance at a 90-day SRT between days 110–130 in S4. Data in Huang et al. [33] indicated a higher SMP_{MLSS} value at longer SRTs, in contradiction to the outcomes both of this study at 20 °C and of the earlier research at 36 °C [32]. While the values reported by Huang et al. [33] did not include dissolved CH_4, the same trend was repeated for all trials at a given HRT, whereas in theory, the effluent methane content in the effluent should be the same in each case. The absence of steady-state conditions under which stable MLSS concentrations can be achieved makes it difficult to identify reliable values for normalised SMP from other similar studies. For higher-strength wastewaters in mesophilic conditions, however, the same trends can be seen as in the current work. During AnMBR treatment of thin corn stillage, the SMP_{MLSS} of 0.016 L CH_4 g^{-1} COD_{rem} L g^{-1} MLSS at day SRT of 20 and 30 days was higher than that of 0.010 L CH_4 g^{-1} COD_{rem} L g^{-1} MLSS for a 50-day SRT [29], while for dairy wastewater 20 and 40-day SRT gave SMP_{MLSS} values of 0.046 and 0.026 L CH_4 g^{-1} COD_{rem} L g^{-1} MLSS, respectively [31].

3.2. Membrane Performance and Fouling Phenomena

This work at 20 °C showed that shorter SRT resulted in better performance with respect to flux but indicated that there was no clear advantage in operating at SRT of <30 days (Figure 2b). In EP-1, however, a more rapid increase in TMP was needed to maintain the flux at a 45-day SRT in S2 and a 60-day SRT in S3 (Figure 2c), compared to that required in S4 at a 90-day SRT, despite considerably higher MLSS concentration in the latter (Figure 2d). This suggests that the onset of membrane fouling was slower at SRT of 30 and 90 days than at 45 or 60 days. In EP-2, the required TMP at a 45-day SRT in S2 was above that for a 90-day SRT in S4 until day 120, while the TMP in S3 began rising sharply from day 106, despite the introduction of a 30-day SRT on day 100, indicating that performance was better at the longer 90-day SRT. TMP in S4 with a 90-day SRT started to increase slightly from day 80, although it was only after day 120 that it began to rise at a rate similar to that seen previously in S3 with a 60-day SRT (Figure 2c). When SRT in S3 and S4 was reduced to 30 days, this led to a complete recovery in flux to the target value of 4 L m^{-2} h^{-1} (Figure 2b), together with reductions in required TMP. Faster recovery was seen in S4, however, most likely because of the abrupt drop in MLSS compared to the slower transition in S3. It should also be noted that the SAnMBR were operated with in situ gas cleaning only through the experimental period, with no external or chemical cleaning. These responses thus not only demonstrate the considerable effect of SRT on membrane fouling but also show that fouling of this type can be at least partially reversed if an optimal SRT is applied.

The above results confirmed that the effect of SRT on membrane fouling is not simply due to the corresponding changes in MLSS concentration. Other research shows that fouling is also affected by components such as EPS, the production of which is strongly related to microbial growth and hence to SRT [3,19]. Research into the effects of SRT on fouling and overall performance in AnMBR is still scarce, however, and the interactions between multiple different factors are often unclear. Work by Huang et al. [33] with synthetic wastewater as well as prior work at 36 °C [32], found that membrane fouling was more severe at longer SRTs. When treating real sewage, however, Huang et al. [34] obtained the highest flux rate at a 60-day SRT, with more severe fouling at both longer and shorter SRTs.

3.3. Mixed Liquor Characteristics

3.3.1. Capillary Suction Time

Results of CST measurements are given in Figure 4a and Table A1. CST values ranged between 94–569 s, and SRT had a strong effect on the mixed liquor's ability to retain moisture: samples taken from SAnMBR operating at shorter SRT released liquid much more readily than those at longer SRT. During the start-up period, when all four SAnMBR were operating as replicates at a 90-day SRT, the increase in CST was similar in all cases. Differences in CST began to appear in EP-1, with values generally higher at longer SRT. By the end of EP-1, however, CST values in S3 and S4 were similar at 438 and 430 s, respectively, despite a 25% difference in MLSS concentrations. This result indicates that the 60-day SRT in S3 represented the least favourable conditions for moisture removal and is reflected in the fall in membrane flux during this period despite continuing increases in TMP. After SRT in S3 was reduced to 30 days at the start of EP-2, the CST began to fall. The value of CST per unit of MLSS (CST_{MLSS}, Figure 4b) continued to rise, however, showing that normalised mixed liquor filterability was still dropping. At the end of EP-2, the CST_{MLSS} value in S3 was twice that in S4, although S3 was operating at one third of the SRT in S4.

At the start of EP-3, SRT was reduced to 20 days in S1 and to 30 days in S4, in conjunction with a sharp reduction in MLSS content in the case of the latter. While CST values in S1 fell gradually, reaching around 100 s by the end of the phase, the sharp MLSS reduction in S4 produced a much steeper decline in CST, which fell from over 550 to 259 s by day 207 then stabilised at around 250 s. CST values continued to fall in S3, while in

contrast, in S2 the CST rose significantly, stabilising at around 480 s, in parallel with a major fall in flux rate.

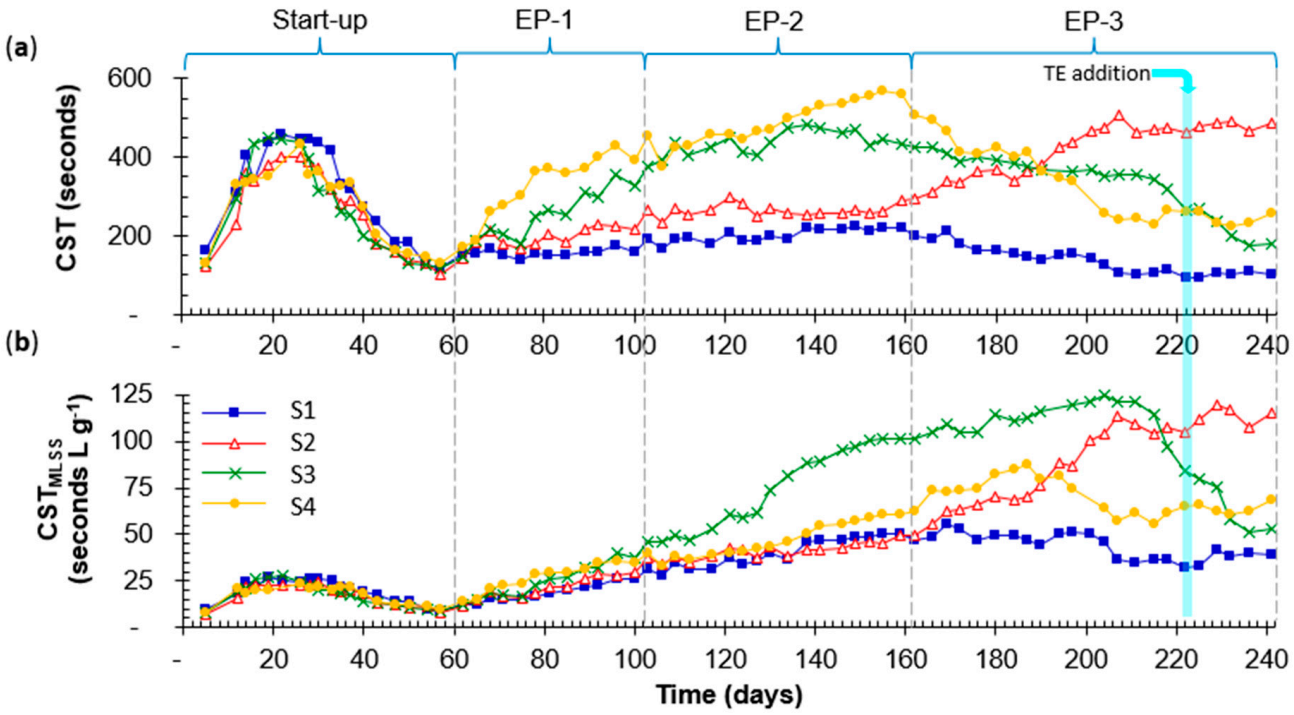

Figure 4. Mixed liquor filterability in SAnMBRs during the whole experimental period: (**a**) CST; (**b**) CST_{MLSS}. Vertical dotted lines indicate the start of experimental phases. Values for control parameters (SRT and TMP) in each phase are shown in Figure 2 and Table A2.

The sharp MLSS reduction in S4 at the start of EP-3 resulted in an almost-immediate rise in CST_{MLSS} values, indicating an increase in normalised filterability of the mixed liquor. This was reflected in the observed recovery in flux, which allowed significant reductions in the required TMP (Figure 2). In contrast, although CST values in S3 levelled off and then began to decrease after SRT was reduced to 30 days in EP-2, it was a further 100 days before the CST_{MLSS} started to fall. A reduction in SRT evidently produces only a gradual change in MLSS characteristics, as time is required for any 'fouling substances' present or microbial species particularly responsible for their production to be eliminated from the reactor. Conversely, if the MLSS concentration is decreased abruptly at the same time as SRT is reduced (e.g., by replacing a proportion of the mixed liquor as in S4), MLSS filterability can improve almost instantaneously as fouling materials are removed or disrupted, and the enhancements in CST and flux occur much more rapidly.

The steady-state CST values of around 100, 480 and 190 s in S1, S2 and S3 operating at 20, 45 and 30-day SRTs, respectively, at the end of EP-3 was higher than those generally reported for aerobic membrane bioreactors (MBR) treating municipal wastewater, which are typically on the order of 10 s [54,55]. CST_{MLSS} values in S1, S2 and S3 ranged from 39 to 113 s L g^{-1} MLSS, again higher than the normalised values of around 1–2 s L g^{-1} MLSS for aerobic MBR. CST and CST_{MLSS} values for combined or co-mingled primary and secondary sewage sludges show more variability but typically range from 50–200 s and 2–10 s L g^{-1}, with digestion sometimes leading to an increase in one or both parameters [56–58]. CST values of around 70 s were reported for MLSS from a full-scale SAnMBR treating source-separated blackwater in Spain [59], with the corresponding CST_{MLSS} of around 15–17 L g^{-1} MLSS, both similar in scale to those found in the current work. Dong et al. [25] operated a pilot-scale AnMBR on screened municipal wastewater at 23 °C at SRT of 40, 70 and 100 days, and reported a reduction in CST at the shorter SRT, although the system did not reach steady-state in each case. A similar pattern of reduction in CST at shorter SRT was

also noted in a cross-flow AnMBR operating at 37 °C on thin corn stillage [29]. The authors reported that CST_{MLSS} values were similar at a 30 or 50-day SRT, and lower at 20 days, but again did not complete a full three SRT in all conditions.

3.3.2. Frozen Image Centrifugation

Frozen Image Centrifugation was developed by the UK's Water Research Centre to allow assessment of sludge dewaterability and thickening characteristics [60]. Although it has never been widely adopted in the water industry, it provides the basis for approaches to the design and operation of dewatering facilities [61,62].

Results from FIC testing are shown in Figure 5a, with the centrifuged sludge volume expressed as a percentage of the original MLSS sample at one-minute intervals during the FIC test. Values normalised against the original MLSS concentration are shown in Figure A2 in Appendix A. Similar to the CST values, these FIC results show clearly that SRT had a significant effect on solid-liquid separation, with MLSS samples taken at short SRT much more readily separable than those at longer SRT. As can be seen from Figure 5a, the rate of separation in the first few minutes of centrifugation was also considerably more rapid in SAnMBRs at shorter SRT, and the final sludge volumes were achieved in a shorter time. At the end of EP-3 the final sludge volumes for FIC tests were 13%, 18% and 29% at 20, 30 and 45-day SRT in S1, S3 and S2, respectively, while the corresponding final centrifuged solids concentrations were 20.1, 19.6 and 14.9 mg L^{-1} and separation rates in the first minute were 5.25, 3.75 and 0.50 mm min^{-1}. After one minute of centrifugation, S1 and S3 were close to their final volumes, while S2 was still compacting at the end of the test. Similar values at a 30-day SRT were also found in S1 at the end of EP-2 and S4 at end of EP-3, indicating some replicability of results.

One benefit of the FIC test is its ability to provide information on several aspects of dewaterability, all of which are potentially important with respect to equipment design and operating costs. The potential for a high ultimate solids concentration, for example, may be less significant if the time required to achieve it is inordinately long [62]. FIC can also reveal details and nuances not readily observable in other tests. In the current work, for example, from day 154 onwards, three separate phases were seen in many of the test samples. These consisted of a clear supernatant, then a distinct cloudy white layer overlying the darker layer of centrifuged sludge solids (Figure 5b). Times at which these layers were present are indicated by solid bar-colouration in Figures 5a and A1, and it can be seen that they remained visible for longer in samples taken from the SAnMBRs operating at longer SRT. The proportion of the test run during which three phases could be distinguished increased in S2 at 45-day SRT, but fell in S3 where the SRT had been reduced from 60 to 30 days, corresponding to improvements in TMP and CST. The appearance of the third phase of this type was also noted in FIC tests on digestate from sugar beet pulp [63]: it was referred to as the light fraction and constituted a high molecular weight material most probably composed of EPS and/or soluble microbial products. Its presence was more evident during periods of foaming, which, such as poor dewaterability, is associated with high sludge viscosity and EPS content. After the change from 60- to 30-day SRT in S3 at the end of EP-1, the CST remained high while final sludge volumes in the FIC test fell. Normalised values of both parameters continued to rise for almost 100 days, however, before dropping quite sharply around day 211. In S4 where the change to 30-day SRT at the end of EP-2 was achieved by MLSS dilution, a sharp fall in CST_{MLSS} after day 189 corresponding to a rapid increase in the separation rate at the start of the FIC test, matched by a reduction in time needed to reach the final volume. The reasons for these delayed but sudden shifts are unknown: they may be related to the passing of a threshold concentration for some component in the MLSS, or even to quorum behaviour of MLSS and biofilm organisms, a topic of growing interest for AnMBR performance and fouling [10,64].

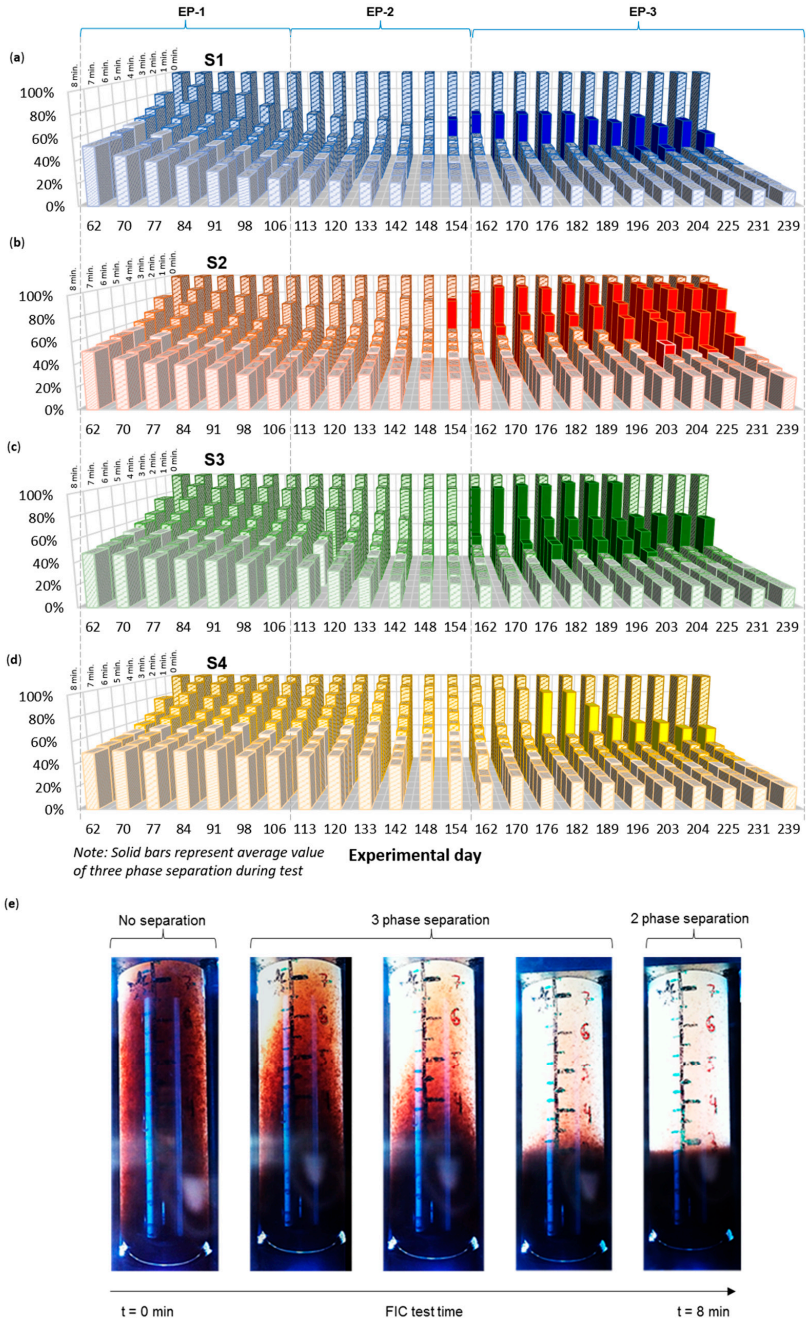

Figure 5. Mixed liquor centrifugation during experimental period: FIC test—Separated sludge volumes as % of original sample volume in (**a**) S1, (**b**) S2, (**c**) S3 and (**d**) S4, (**e**) separation phases during FIC test in centrifuged mixed liquor from S2 on day 204. Vertical dotted lines indicate start of experimental phases.

Several researchers have investigated relationships between parameters such as CST or specific resistance to filtration (SRF) and MLSS dewaterability, membrane flux and fouling in both aerobic and anaerobic MBR [25,29,59,65]. CST is generally considered to be one of the most useful parameters because it shows a reasonably good correlation with membrane performance and is relatively easy to evaluate on-site [27,35]. In the current work, regression analysis was carried out to investigate any relationship between applied TMP (as an indicator of filterability and membrane fouling) with CST and FIC. However, in both cases, interpretation was made difficult by periods at maximum TMP. Although neither showed a very strong correlation, the relationship between TMP and CST was generally stronger than for TMP and FIC. This is as might be expected as the

water removal mechanism in a CST test where liquid is drawn through the MLSS is more similar to that in membrane bioreactor than in FIC. Several authors have noted the importance of using a type of test, which reflects the dewatering technology [66,67], and FIC testing has greater similarity to both sludge thickening and centrifugation. The value of expanding the range of tests currently utilised for sludge characterisation was emphasised by Spinosa and Doshi [68], and further work on FIC and other tests is needed to provide an enhanced understanding of different parameters and the significance of the relationships between them.

3.3.3. Extracellular Polymeric Substances

Under steady-state conditions at the end of EP-3, bound EPS concentrations at 20, 30 and 45-day SRT were 378, 483 and 511 mg L^{-1} in S1, S3 and S2, respectively. Specific values normalised against MLSS were around 165 mg g^{-1} VSS for the SAnMBR at 20 and 30-day SRT, and slightly lower at 134 mg g^{-1} VSS at a 45-day SRT. Other authors have also reported higher EPS content at shorter SRT: examples include treatment of municipal wastewater in a sidestream AnMBR at SRT ranging from 19–217 days, although steady-state operation was achieved only at the 19-day SRT [22]; and of synthetic wastewater at 31 °C in a AnMBR with a ceramic membrane at 100, 50 and 25-day SRT [69], although with full steady-state operation for the 25-day SRT only.

As can be seen in Figure 6a,b, for much of the experimental period, the specific concentrations of bound protein and carbohydrate were highest in S1 operating at a 30-day and 20-day SRT. When SRT in S3 was reduced from 60 to 30 days at the start of EP-2, however, specific concentrations increased in this reactor, with the carbohydrate content rising first. Specific protein and carbohydrate concentrations also increased in S4 after the SRT was reduced to 30 days at the start of EP-3. At the end of the experimental period, both the specific bound protein and the bound carbohydrate concentrations were lowest in S4 at a 45-day SRT, and higher in the SAnMBR at shorter SRT. Huang et al. [33,34] found higher specific protein contents in EPS at a 30-day SRT than at longer SRT, and suggested that this might produce larger flocs with a lower membrane fouling propensity. They also noted that long SRTs were associated with smaller median particle size, as the lower EPS content reduces flocculation, and hence promotes more rapid fouling. These suggestions appear to be consistent with the outcomes of the current work.

There was also evidence of a relationship between soluble EPS content and membrane performance at longer SRTs. As can be seen in Figure 6c,d, the specific carbohydrate and protein concentrations of soluble EPS in S3, which had shown the most rapid fouling of all at a 60-day SRT, were generally higher than in the other SAnMBRs. After SRT in S3 was reduced to 30 days from the start of EP-2, the specific soluble protein and carbohydrate contents continued rising until around day 205 when they finally reduced. At that point, the CST value also fell, the rate of separation in the FIC test increased, and the flux began to recover. Conversely, towards the end of EP-3, the specific soluble protein and carbohydrate contents in S2, operating at a 45-day SRT, increased relative to the other SAnMBRs, which at that point were all running at shorter SRTs. This corresponded to the period in which CST values in S2 began to rise and flux to reduce, despite an increase in TMP, to 9.8 kPa. This reactor reached the end of the experiment with the highest soluble protein and carbohydrate contents (specific and absolute), highest TMP, highest CST and FIC values, lowest separation rate in the FIC test and lowest flux.

Huang et al. [33] found that the concentration of soluble microbial products was inversely related to SRT, while higher SMP carbohydrate and protein contents at longer SRT resulted in higher fouling rates. In contrast, when real municipal wastewater feed was used, the minimum specific and absolute values for SMP carbohydrate and proteins were found at a 60-day SRT [34]. This was attributed to incomplete substrate degradation at the 30-day SRT, and an increased concentration of residual cell materials at the 90-day SRT. Although EPS and MLSS concentrations were lowest at the shortest SRT, this was insufficient to counteract the fouling effect of the higher SMP content. Soluble microbial

products were not measured in the current study, but this explanation may be partly supported by the results of this experiment and of the previous work at 36 °C [32], which showed no further enhancement in membrane performance at SRT of <30 and <25 days, respectively. Laspidou and Rittmann [70] proposed that under some conditions soluble EPS and SMP may be similar, although other researchers were unable to confirm this [71]. Trends in bound and soluble EPS observed in this work are consistent with those in other studies carried out under steady-state conditions, where the consensus is that bound EPS tends to be high at shorter SRTs while microbially-induced SMP concentrations tend to decrease [33,34]. Given that soluble EPS also decreased at shorter SRTs in the current work, it can be concluded that even if it is not identical to SMP, its relationship to SRT and its effects on membrane fouling are closely similar.

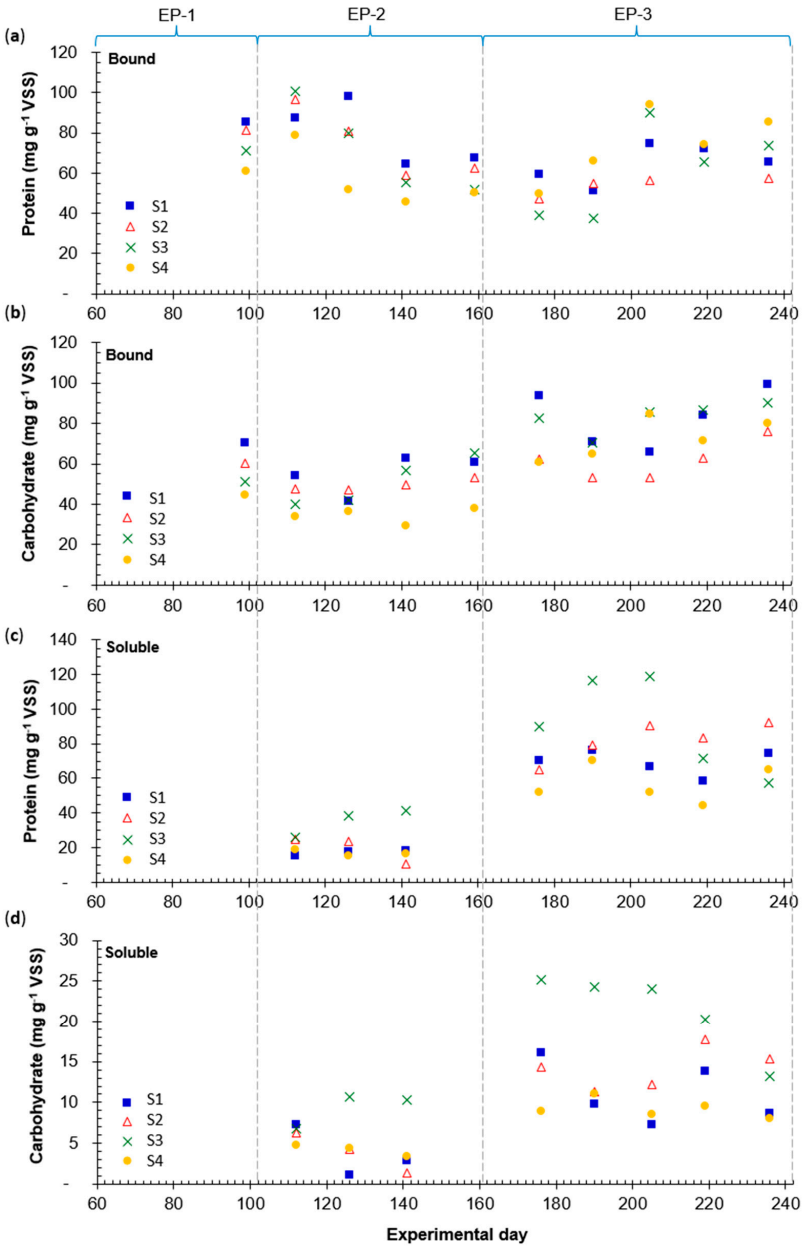

Figure 6. EPS composition during experimental period: (**a**) bound protein, (**b**) bound carbohydrate; (**c**) soluble protein, (**d**) soluble carbohydrate. Vertical dotted lines indicate start of experimental phases. Values for control parameters (SRT and TMP) in each phase are shown in Figure 2 and Table A2.

EPS content was not measured in the previous work with the same wastewater at 36 °C [32], and thus the effect of temperature in the current study is unknown. EPS and soluble microbial products normalised to MLSS content were reported to increase with decreasing temperature in a flat-sheet SAnMBR fed on a synthetic municipal wastewater at 25, 15 and 10 °C [14]. The ratio of protein to carbohydrate also rose with decreasing temperature, leading to higher rates of fouling at low temperatures. The applied SRT was not reported, however, thus it is likely that this was high and determined only by the withdrawal of samples for analysis. Similar trends in EPS and soluble microbial product concentrations were observed in two hollow fibre SAnMBR fed on synthetic wastewater at 25 and 35 °C with an SRT of 370 days [72], and in a hollow fibre SAnMBR fed on municipal wastewater at 25, 20 and 15 °C at SRT of 93.9, 40.3 and 20.7 days, respectively [73], although the operating periods under each set of conditions were less than 3 SRT. Kong et al. [1] operated a 5 m^3 pilot-scale SAnMBR with a HFM unit on municipal wastewater for over 200 days at 25 °C. No change in EPS concentration was observed when HRT was varied between 6–24 h, but SRT was not reported.

EPS and soluble microbial products were characterised in flat-sheet SAnMBR treating low-strength synthetic wastewater at 25 °C and operated at various OLR [19]. Properties were linked to membrane fouling mechanisms, but the SRT of the system was near-infinite as no MLSS was discharged other than for sampling.

While some differences in EPS production and composition were seen in the current study, these were not sufficiently marked enough to show a clear correlation between SRT and observed fouling behaviour. Nonetheless, the findings are consistent with those reported elsewhere. While there may be a specific range of SRT for each system in which membrane performance can be optimised, however, the effect of EPS concentrations and compositions on fouling in AnMBR is still not well understood, as a function of SRT or other operating parameters. For this reason, further work may be needed to assess the interaction between operational variables, MLSS characteristics and membrane fouling.

3.3.4. Biomass Growth and Kinetics

As well as having a significant impact on mixed liquor characteristics, the MCRT or SRT controls microbial growth rates, and thus potential growth yield. As previously seen for the work carried out at 36 °C [32], in the current experiment, the biomass yield appeared to show a sharp increase at each reduction in SRT. This reflected the fact that, as noted in the Methods section, changes in stored biomass were not taken into account to eliminate major variations. The apparent increases in yield were followed by a gradual decrease as conditions stabilised. The stable value was taken as the representative yield for each corresponding SRT and was equal to 0.131 ± 0.008 g VSS g^{-1} COD_{rem} at a 20-day SRT, 0.124 ± 0.012 g VSS g^{-1} COD_{rem} at a 30-day SRT, and 0.114 ± 0.004 g VSS g^{-1} COD_{rem} at a 45-day SRT. Biomass yields for the 60- and 90-day SRTs are not reported here as steady-state operation of the SAnMBRS was not achieved for these conditions, and thus representative values were not available.

The above results confirmed that shorter SRT gave higher biomass yields, in agreement with prior work at 36 °C [32]. These outcomes provide further support for the idea that at shorter SRT a higher proportion of the available carbon is directed into biomass growth rather than methane production, thus reducing the substrate SMP. Conversely, at longer SRT, lower growth rates and increased hydrolysis of MLSS leads to reduced biomass yields and higher SMP values [24]. In a full-scale system, a shorter SRT with a higher biomass yield would be linked to larger volumes of waste sludge for disposal and potentially to a greater requirement for TE supplementation, both leading to higher operating costs.

These low yields are typical of those reported elsewhere for similar systems, including 0.11 g VSS g^{-1} COD for low-strength synthetic wastewater treatment at 25 °C and near-infinite SRT [74]. Ji et al. [73] found sludge yields of 0.12, 0.19 and 0.388 g VSS g^{-1} COD_{rem} with real municipal wastewater at SRT of 93.9, 40.3 and 20.7 days; but the effect was confounded by accompanying changes in temperature from 25 to 20 and 15 °C and the

system did not operate for 3 SRT in each set of conditions. Sludge yields of 0.07–0.11 g VSS g^{-1} COD_{rem} were reported using the same wastewater at 25 °C for SRT from 65 days to near-infinite with HRT from 6–12 h and OLR between 0.7–1.5 g COD L^{-1} day^{-1} [28].

The current study showed changes occurring over different timescales, which could be categorised as follows: (i) those which happen slowly, such as stabilisation of MLSS concentrations after a change in SRT; these changes cannot easily be accelerated as they are both growth-mediated and affected by washout rate, although interventions such as partial removal of MLSS may reduce the time needed to approach stable values. (ii) Those which can happen more rapidly, such as a response to TE addition or to other factors affecting the microbial population; as these are metabolically mediated, they can trigger an immediate response through stimulation or inhibition. (iii) Those which may happen rapidly but after a delay, such as the observed sharp changes in CST and FIC values following reductions in SRT, which could be metabolically or physico-chemically determined in relation to threshold concentrations. The factors that cause these changes may also be interdependent, and hence to allow full evaluation of their effects on system performance and mixed liquor characteristics long-term operation is advisable, wherever possible for at least 3 SRT under a given set of operating conditions.

4. Conclusions

Extended operation of four SAnMBRs at 20 °C on a low-to-intermediate strength substrate with a high suspended solids content was conducted at different SRTs. This enabled accurate determination of flux rates at specific SRT, accompanied by evaluation of COD removal efficiencies; estimation of biomass yields; a COD-based mass balance; physical characterisation of mixed liquors using CST and FIC tests; and analysis of EPS concentration, type and composition. The results showed that SRT had a considerable effect on flux rates, with shorter SRT allowing enhanced membrane performance and improved mixed liquor filterability, at a higher bound EPS content but with lower soluble EPS concentrations in the MLSS. Operation at shorter SRT led to a reduction in specific methane productivity and in COD removal rates, accompanied by higher biomass yields. Whilst no further enhancement of membrane performance was found at SRT of <30 days, operation at a 60-day SRT resulted in more rapid onset of membrane fouling and declining performance than at a 90-day SRT. Reduced COD removal rates at shorter SRTs were probably due to the increased washout of essential trace elements caused by the higher biomass turnover: this was supported by a rapid recovery in COD removal efficiency after TE supplementation was carried out. COD removal efficiencies remained slightly lower at shorter SRTs, however, suggesting that the lower available biomass concentration may also have affected this parameter. Overall COD removal efficiencies achieved after TE addition were very close to those seen in an earlier study using the same substrate at 36 °C, and the effect of the lower operating temperature on this parameter was, therefore, considered to be negligible. CST values gave some indication of changes in membrane performance, while frozen image centrifugation provided additional insights into MLSS properties and fouling behaviour, with three separate phases clearly visible at longer SRT. The ability of the FIC test to identify several parameters such as final solids concentration and rates of solid separation may also make it especially appropriate for assessing sludge dewaterability in gravity thickening or centrifugation, and further exploration of this approach is recommended. Operation over the full experimental period, without chemical or external cleaning, not only demonstrated the effects of SRT on performance parameters but also indicated that membrane fouling could be at least partially reversed if an optimal SRT is applied. These results indicated that responses to a change in SRT may be significantly delayed, probably as a result of the different timescales on which growth-related, metabolic and physico-chemical and/or the various interactions between them. Thus confirming the importance of long-term operation to allow full evaluation of system performance and mixed liquor characteristics under steady-state conditions. Together with earlier findings from operation at 36 °C, this work confirmed that there are potential trade-offs to be made between

membrane performance, specific methane productivity and sludge yields when selecting a suitable SRT for AnMBR systems of this type.

Author Contributions: Conceptualization and methodology, S.P.-R., S.H., C.J.B.; experimental work, S.P.-R.; data analysis and interpretation, S.P.-R., S.H., C.J.B.; writing—original draft preparation, S.P.-R.; writing—review and editing, S.H., C.J.B.; supervision, C.J.B., S.H.; funding acquisition, C.J.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Mexican National Council on Science and Technology (CONACYT), the Faculty of Engineering and Physical Sciences at the University of Southampton, and the BBSRC ERA-Net AmbiGAS project BB/L000024/1.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are openly available on DOI: 10.5258/SOTON/D1950.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

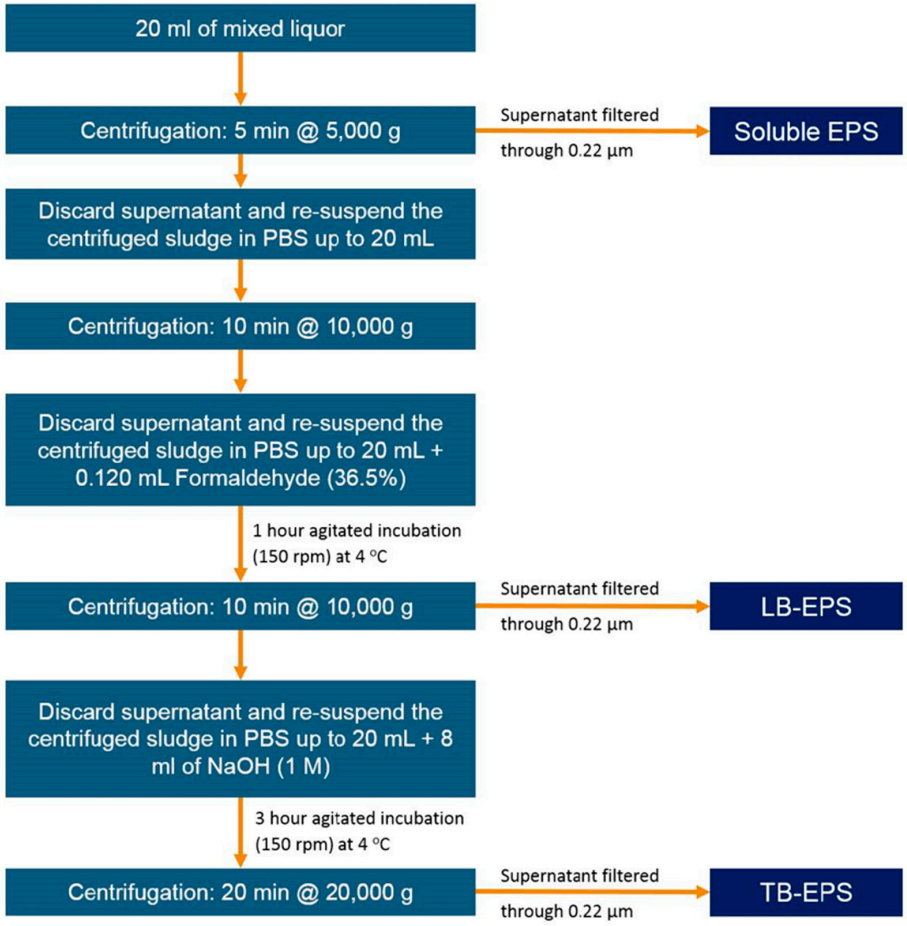

Figure A1. Flowchart for EPS extraction procedure.

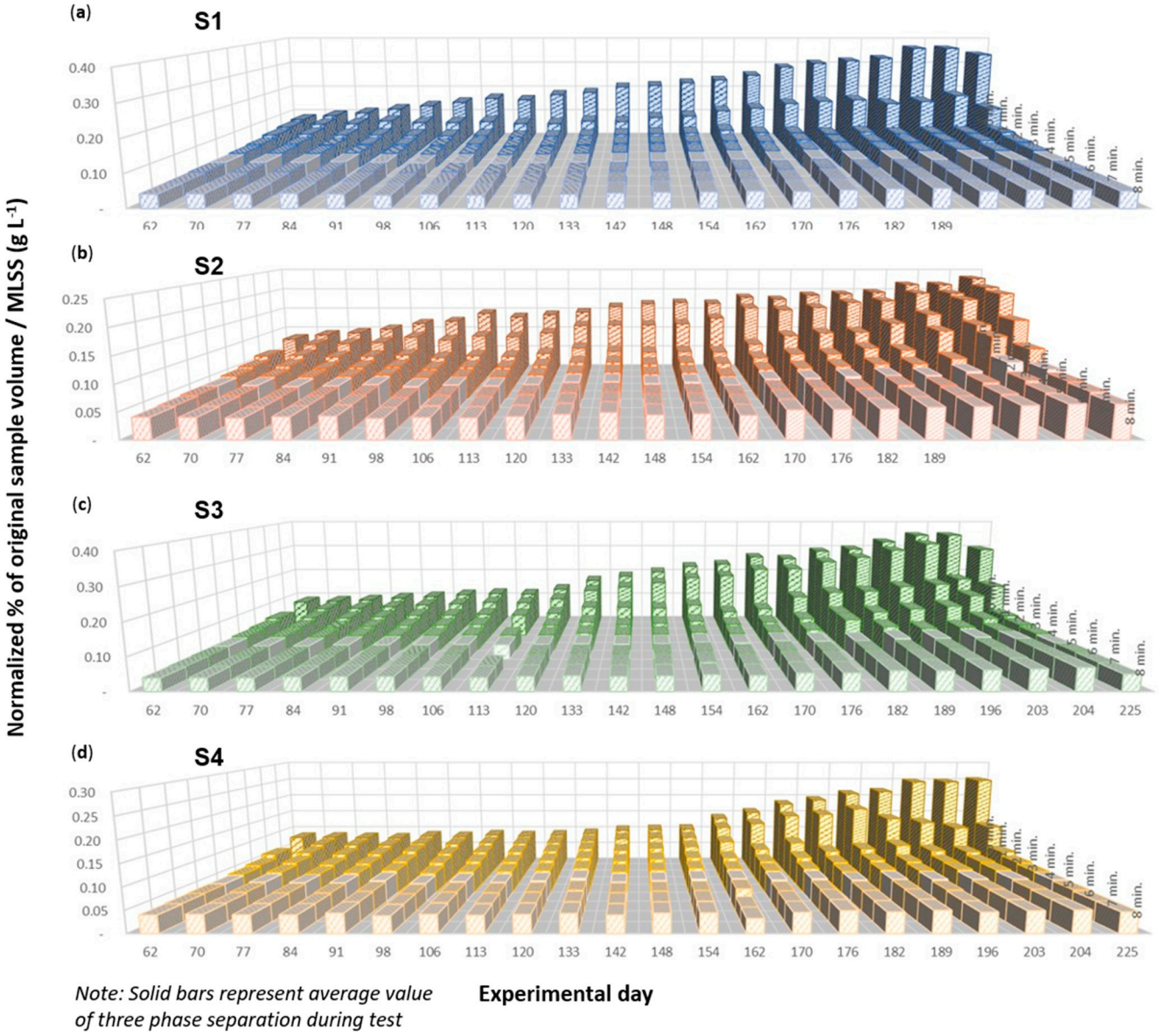

Figure A2. FIC test sludge volumes results normalised to MLSS content in (**a**) S1, (**b**) S2, (**c**) S2 and (**d**) S4.

Table A1. Experimental results; SRT effect on: membrane flux, OLR, HRT, feed COD, effluent COD and COD removal rates.

Parameter / Phase	Reactor	SRT (days)	TMP (kPa)	Membrane Flux (J) * (L m^{-2} h^{-1})	OLR (g COD L^{-1} day^{-1})	HRT (hours)	Feed COD (mg L^{-1})	Effluent COD (mg L^{-1})	COD Removal Rate (%)
Start-up (day 0–59, total 60 days)	A	90	7.1 → 2.1 → 2.5	16.1 → 6.7	0.50–1.45	5.8 → 12.5	305 → 507	99 → 200 → 59	74% → 53% → 89%
	B	90	7.1 → 2.1 → 2.5	16.1 → 6.4	0.50–1.47	5.8 → 13.1	305 → 507	100 → 222 → 51	74% → 55% → 90%
	C	90	7.1 → 2.1 → 2.5	16.1 → 6.3	0.50–1.49	5.8 → 13.3	305 → 507	83 → 213 → 39	79% → 49% → 93%
	D	90	7.1 → 2.1 → 2.5	16.1 → 6.4	0.50–1.46	5.8 → 13.1	305 → 507	83 → 224 → 62	72% → 42% → 88%
EP-1 (day 60–111, total 52 days)	A	30	2.5 → 3.2 → 1.8	6.7 → 5.3 → 4.1	0.98 ± 0.03	12.5 → 15.8 → 20.2	567 → 860	59 → 144	88% ± 1%
	B	45	2.5 → 5.1 → 2.9	6.4 → 5.1 → 4.1	0.94 ± 0.04	13.1 → 16.5 → 20.3	567 → 860	51 → 65	91% ± 1%
	C	60	2.5 → 6.7 → 4.3 → 6.3	6.3 → 4.2 → 3.7	1.00 → 0.90	13.3 → 17.7 → 22.5	567 → 860	41 → 36	92% ± 2%
	D	90	2.5 → 3.2 → 2.2	6.4 → 5.3 → 4.1	0.97 ± 0.02	13.1 →16.2 → 20.4	567 → 860	61 → 21	88% → 97%
EP-2 (day 112–160, total 48 days)	A	30	1.8 ± 0.5	4.1 ± 0.0	0.98 ± 0.01	20.6 ± 0.2	843 ± 26	144 → 125	85% ± 2%
	B	45	2.8 ± 0.6	4.1 ± 0.0	0.99 ± 0.01	20.5 ± 0.2	843 ± 26	65 → 70	93% ± 1%
	C	30	6.3 → 9.8	3.7 → 3.0	0.90 → 0.72	22.5 → 28.5	843 ± 26	36 → 77	95% → 91%
	D	90	2.2 → 9.8	4.1 → 3.2	0.97 → 0.76	20.4 → 26.7	843 ± 26	21 → 44	96% ± 1%
EP-3 (day 161–242, total 82 days)	A	20	1.9 → 2.2	4.0 ± 0.1	0.98 ± 0.02	21.0 ± 0.4	853 ± 21	136 → 228 → 68	84% → 74% → 92%
	B	45	2.8 → 9.8	4.1 → 3.2	0.99 → 0.78	20.5 → 26.3	853 ± 21	70→ 84 → 22	92% → 92% → 97%
	C	30	9.8 → 3.8	3.0 → 2.7 → 4.0	0.72 → 1.02	28.5 → 31.5 → 21.9	853 ± 21	77 → 217 → 39	91% → 75% → 96%
	D	30	9.8 → 6.5	3.2 → 4.1	0.76 → 0.99	26.7 → 21.1	853 ± 21	44 → 91 → 34	95% → 89%→ 96%

(→) Variable trend: initial → middle → final; (±) Stable performance: One standard deviation to show spread of data from the average value under stable performance; (*) Daily average.

Table A2. Experimental results; SRT effect on: CH_4 content in biogas, SMP, MLSS, MLVSS, CST and pH.

Parameter		SRT	CH_4 in Biogas *	SMP **	MLSS	MLVSS	CST	
Phase	Reactor	(days)	(%)	(L CH_4 g^{-1} COD Removed)	(g L^{-1})	(g L^{-1})	(seconds)	pH
Start-up (day 0–59, 60 days)	A	90	88% → 93%	0.00 → 0.35 → 0.21	16.2 → 12.7	12.5 →10.3	162 → 461 → 117	7.0 → 6.2 → 6.8
	B	90	81% → 92%	0.00 → 0.29 → 0.20	16.2 → 12.5	12.6 → 10.2	122 → 402 → 101	7.0 → 6.2 → 6.8
	C	90	76% → 93%	0.00 → 0.42 → 0.22	16.2 → 12.4	13.3 → 10.0	130 → 449 → 117	7.0 → 6.2 → 6.8
	D	90	87% → 93%	0.00 → 0.42 → 0.21	16.2 → 12.7	12.7 → 10.4	132 → 435 → 131	7.0 → 6.2 → 6.9
EP-1 (day 60–111, 52 days)	A	30	92% ± 1%	0.21 ± 0.01	12.7 → 6.2	10.3 → 5.4	117 → 198	6.8 ± 0.0
	B	45	91% ± 1%	0.22 ± 0.02	12.5 → 7.3	10.2 → 6.2	101 → 252	6.8 ± 0.0
	C	60	92% ± 2%	0.23 ± 0.02	12.4 → 8.7	10.0 → 7.4	117 → 438	6.8 ± 0.0
	D	90	92% ± 2%	0.23 ± 0.02	12.7 → 11.7	10.4 → 9.8	131 → 430	6.8 ± 0.0
EP-2 (day 112–160, 48 days)	A	30	90% ± 1%	0.22 ± 0.01	6.2 → 4.4	5.4 → 3.8	198 →215	6.7 ± 0.0
	B	45	90% ± 1%	0.24 ± 0.01	7.3 → 6.0	6.2 → 5.2	252 →291	6.7 ± 0.1
	C	30	90% ± 1%	0.23 ± 0.01	8.7 → 4.3	7.4 → 3.7	407 → 482 → 433	6.7 ± 0.0
	D	90	90% ± 1%	0.26 ± 0.02	11.7 → 9.3	9.8 → 8.1	430 → 569	6.8 ± 0.0
EP-3 (day 161–242, 82 days)	A	20	90% ± 1%	0.23 ± 0.01	4.4 → 2.6	3.8 → 2.2	222→ 101	6.7 ± 0.0
	B	45	90% ± 1%	0.25 ± 0.01	6.0 → 4.2	5.2 → 3.7	291 → 488	6.8 ± 0.0
	C	30	90% ± 1%	0.23 ± 0.01	4.3 → 2.8 → 3.4	3.7 → 2.9	433 → 182	6.8 ± 0.0
	D	30	90% ± 1%	0.24 ± 0.01	9.3 → 6.4 → 3.8	8.1 → 3.3	569 → 240	6.8 ± 0.0

(→) Variable trend: initial → middle → final; (±) Stable performance: One standard deviation to show spread of data from the average value under stable performance; (*) Normalised to total biogas content in sample (i.e., neglecting air introduced dissolved through feed); (**) Takes into account the methane dissolved in the effluent.

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