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Biological Desulfurization of Tannery Effluent Using Hybrid Linear Flow Channel Reactors

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Abstract: The tanning process generates a saline effluent with high residual organics, sulfate and sulfide concentrations. The transition from a linear to circular economy requires reimagining of waste streams as potential resources. The organics in tannery effluent have the potential to be converted to renewable energy in the form of biogas if inhibitors to anaerobic digestion are removed. Hybrid linear flow channel reactors inoculated with culture-enriched halophilic sulfate reducing bacteria from saline environments were evaluated as a novel pretreatment step prior to anaerobic digestion for the concurrent removal of sulfur species and resource recovery (elemental sulfur and biogas). During continuous operation of a 4-day hydraulic retention time, the reactors were capable of near-complete sulfide oxidation (>97%) and a sulfate reduction efficiency of 60–80% with the formation of a floating sulfur biofilm containing elemental sulfur. Batch anaerobic digestion tests showed no activity on untreated tannery effluent, while the pretreated effluent yielded 130 mL methane per gram COD consumed.

Keywords: anaerobic digestion; biological sulfate reduction; floating biofilm; sulfide oxidation; sulfur recovery

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1. Introduction

Tanning is the chemical process of transforming animal hides and skin to leather. It is one of the oldest raw material processing techniques in the world and is an important economic activity in many developing countries [1].

The tanning process is chemically intensive and generates large quantities of highly turbid, saline and foul-smelling effluent that is laden with toxic metal salts such as chromium (Cr^{3+}) and suspended solids, as well as organic residues and high concentrations of inorganic ammonium (NH_4^+), sulfates (SO_4^{2-}) hydrogen sulfides (HS^-), sodium (Na^+) and chlorides (Cl^-) [2–4]. Tannery wastewater (TWW) poses an environmental threat, but it is a challenge to treat adequately due to its complexity and qualitative and quantitative variability [2,4].

To meet ever-increasing environmental standards, three or four stages of treatment are required to remediate TWW to comply with legislated discharge criteria worldwide. These include preliminary treatment to screen out coarse material; primary treatment to remove suspended solids, metals and sulfides and secondary treatment to reduce the organic and other macronutrient loads. Secondary processes are typically aerobic biological systems or advanced oxidation systems [3].

During conventional treatment, sulfide management typically occurs as part of the primary stage, with sulfide removed through stripping (H_2S gas), precipitation or oxidation, either by adding hydrogen peroxide (H_2O_2) or through the use of an oxidation catalyst [5]. Although effective, these processes have drawbacks, such as high energy costs, the need for costly gas purification systems for stripping processes, large sludge generation caused

by metal precipitation methods, chemical costs and long reaction times associated with oxidation by H_2O_2 [6,7].

The transition from a linear to more circular way of thinking is a key pillar in the drive toward a more sustainable existence. The high concentrations of organic matter (Table 1) make TWW an attractive substrate for anaerobic digestion (AD) for concurrent generation of energy and reduction in the organic load. However, high S^{2-} concentrations lead to the inhibition of methane (CH_4) generation, due to the particular toxicity of S^{2-} (most notably unionized H_2S) towards functional methanogenic archaeal species [8,9]. Fermentative bacteria are less susceptible to direct H_2S toxicity than acetogenic bacteria, while methanogens are the most affected [9,10]. In addition, the reduction of SO_4^{2-} by sulfate-reducing bacteria (SRB) under anaerobic conditions can lead to further generation of S^{2-}. Further, it has been reported that, during the AD process, SRB may compete with methanogens for simple organic compounds to reduce SO_4^{2-} to HS^-. This competition is influenced by factors such as the chemical oxygen demand (COD):SO_4^{2-} ratio and the microbial community composition [10].

Table 1. Average characteristics of batches (n = 5) of tannery wastewater used in this study.

Parameter	RAW		PARTIALLY TREATED	
	Average	SD	Average	SD
pH	10.24	1.6	7.81	0.52
EC (mS/cm)	32.01	2.2	31.5	1.59
ORP (mV)	−547	61.5	−434	21.6
TOC (mg/L)	6116	1875	886	253
COD (mg/L)	28,169	3665	4968	2940
BOD (mg/L)	6200	812	1539	520
VOA_t (mg/L AAE)	2920	718	1041	647
Protein (mg/L)	2875	1024	310.6	83.9
TN (mg/L)	1258	215	679	138
TAN (mg/L NH_3-N)	301	286	350	235
NO_3^- (mg/L)	70.8	25.0	40.5	23.6
NO_2^- (mg/L)	5.1	5.2	2.35	2.63
PO_4^{3-} mg/L	0	0	1.21	1.96
SO_4^{2-} (mg/L)	1951	574	3687	383
HS^- (mg/L)	699	114	83	76
Cl^- (mg/L)	7744	460	7713	325
TS (g/L)	36.1	5.8	17.96	3.23
TVS (g/L)	13.1	3.3	1.93	0.47
K (mg/L)	95.7	31.1	100.3	19.5
Na (mg/L)	6412	571	6225	276
Fe (mg/L)	0.11	0.08	0.19	0.12
Ca (mg/L)	692	482	230.9	58.5
Mg (mg/L)	120	138	220.7	27.6
Mn (mg/L)	0.50	0.41	15.14	6.92
Zn (mg/L)	0.50	0.33	0.19	0.12
Cr (mg/L)	0.09	0.05	0.20	0.08
Alk (mg/L $CaCO_3$)	3256	907	1999	385
COD:SO_4^{2-}	15.4	3.5	1.4	0.76
TVS:TS	0.36	0.03	0.11	0.02
BOD:COD	0.23	0.05	0.37	0.14
C:N	5.1	2.2	1.3	0.21
VOA:Alk	0.94	0.27	0.48	0.24
COD:TVS	2.2	0.52	2.5	0.93

SD = standard deviation from the mean; EC = electrical conductivity; mS = milliSiemens; ORP = oxidation redox potential; mV = millivolts; TOC = total organic carbon; COD = chemical oxygen demand; BOD = biological oxygen demand; VOA_t = total volatile organic acids; AAE = acetic acid equivalents; TN = total nitrogen; TAN = total ammonia nitrogen; NH_3-N = ammonia nitrogen; NO_3^- = nitrate; NO_2^- = nitrite; PO_4^{3-} = phosphate; SO_4^{2-} = sulfate; HS^- = hydrogen sulfide; TVS = total volatile solids; TS = total solids Alk = alkalinity; $CaCO_3$ = as calcium carbonate; C:N = carbon to nitrogen ratio.

Biological SO_4^{2-} reduction (BSR) is a cost-effective alternative for removing S species from TWW. The reduction of SO_4^{2-} in the presence of a suitable electron donor proceeds via Equation (1), generating HS^- and alkalinity [11]:

$$\text{electron donor} + SO_4^{2-} \rightarrow HS^- + HCO_3^- \quad (1)$$

In an aerobic HS^- removal system, the partial and then complete biological oxidation of HS^-, given by Equations (2) and (3), can take place [12–14]:

$$2\,HS^- + O_2 \rightarrow 2\,S^0 \downarrow + 2\,OH^- \quad (2)$$

$$S^0 + H_2O + \frac{3}{2}\,O_2 \rightarrow SO_4^{2-} + 2\,H^+ \quad (3)$$

Partial oxidation is desirable as the elemental sulfur product (S^0) can be removed from the system, thereby reducing the concentration of S species and preventing SO_4^{2-} accumulation and acidification. This is achievable, provided the oxygen (O_2) availability is carefully controlled to prevent complete oxidation. The majority of integrated processes to remove S species consist of separate reactors where sulfate reduction (SR) and sulfide oxidation (SO) occur sequentially [15].

Marais et al. [16] showed that simultaneous BSR and partial SO is possible in a hybrid linear flow channel reactor (HLFCR) using synthetic SO_4^{2-}-rich water and simple electron donors (lactate and acetate). This is achieved by the separation of reducing (bulk volume) and oxidizing (air–liquid interface) zones due to the hydraulic flow pattern [17]. The authors were able to achieve near-complete SR (1 g/L feed) and 95–100% removal of the resulting S^{2-}, with recovery of a bio-sulfur product that has value as a soil-conditioning agent.

It was hypothesized that HLFCRs may be ideal passive systems for the pretreatment of TWW to reduce S species, recover S^0 and enhance biogas generation during downstream AD. This is the first time that HLFCRs have been evaluated using (i) industrial effluent and (ii) highly saline feed. It is also the first time that AD has been evaluated before and after the pretreatment of TWW using HLFCRs.

2. Materials and Methods

2.1. Microbial Cultures for Inoculation of Hybrid Linear Channel Reactors

Environmental samples were collected from suitable anaerobic water bodies and sediments of varying salinities (electrical conductivity (EC) 35–48 mS/cm) in coastal and estuarine areas of South Africa. Samples were enriched for SRB in artificial sea water (ASW) with the following composition: 23.9 g/L NaCl, 4.01 g/L Na_2SO_4, 0.67 g/L KCl, 0.2 g/L $NaHCO_3$ and 0.03 g/L H_3BO_4. The cultures were screened for SR activity in 20 mL sealed glass bottles with a range of carbonaceous electron donors in ASW. The highest activity was obtained using lactate (1.9 g/L as 60% Na lactate). These saline-adapted cultures containing BSR consortia were subcultured into the same medium in increasingly larger volumes (up to 2 L) and, finally, acclimated to TWW supplemented with lactate for 39 days; after which, they were used as an inoculum during start-up of a HLCFR (Section 2.3).

2.2. Tannery Wastewater

Batches of TWW were provided by a commercial tannery that processes bovine and ovine hides and skins. Batches of two sets of effluent (raw and partially treated) were blended in order to maintain similar pH, SO_4^{2-} and HS^- concentrations in the influent during the experimental period. The characteristics of the TWW are given in Table 1. All analytical procedures were performed as previously described [18].

2.3. Set-Up and Operation of Hybrid Linear Flow Channel Reactors (HLFCR)

The pretreatment experiments were performed using HLFCRs similar to those previously used in experiments to treat synthetic mining-impacted water [16]. The reactor had dimensions of 250 mm (l) × 100 mm (w) × 150 mm (h) (Figure 1).

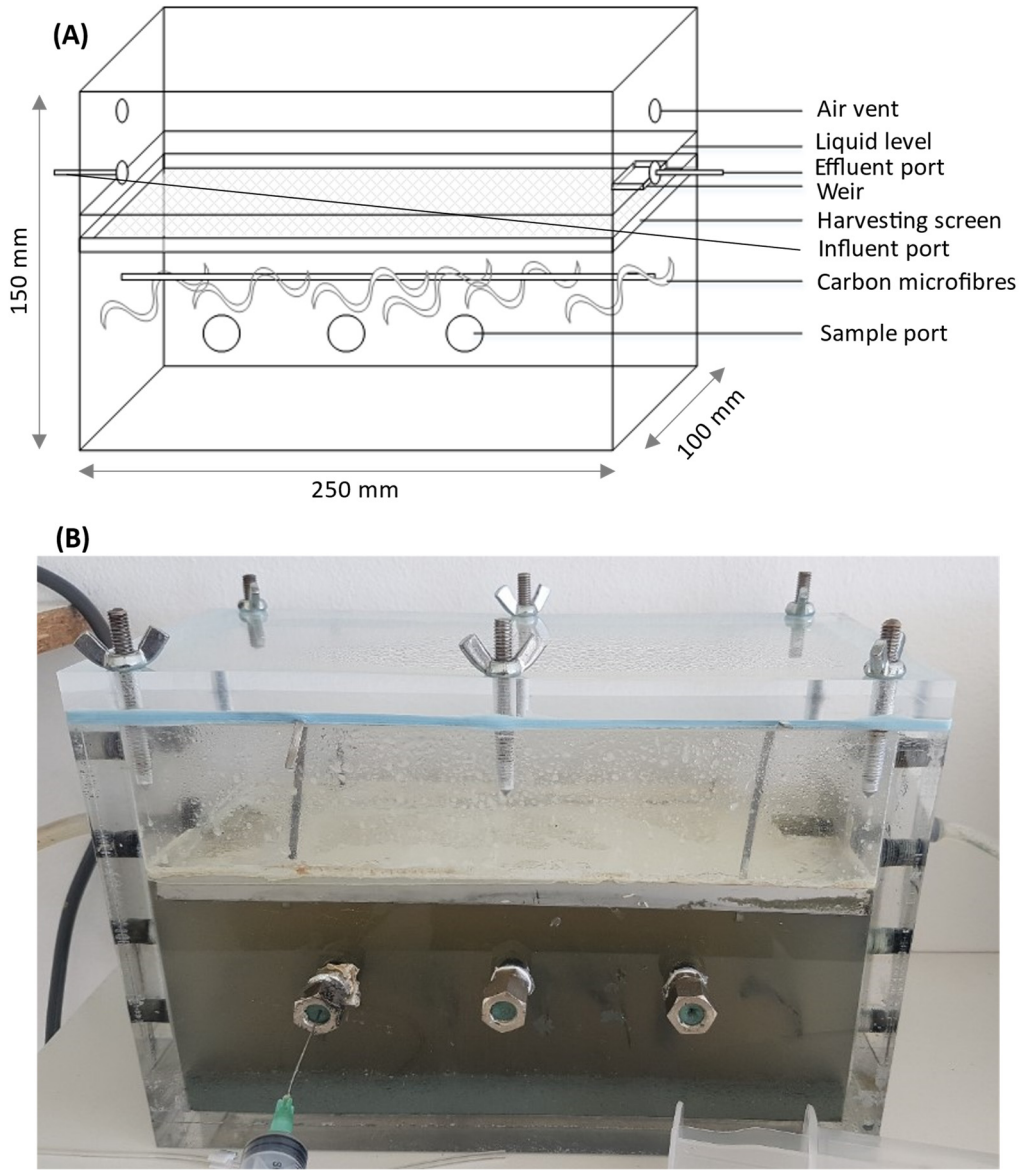


Figure 1. Labeled diagram of a linear channel flow reactor (**A**), and a photograph of this reactor containing tannery effluent with a floating sulfur biofilm (**B**).

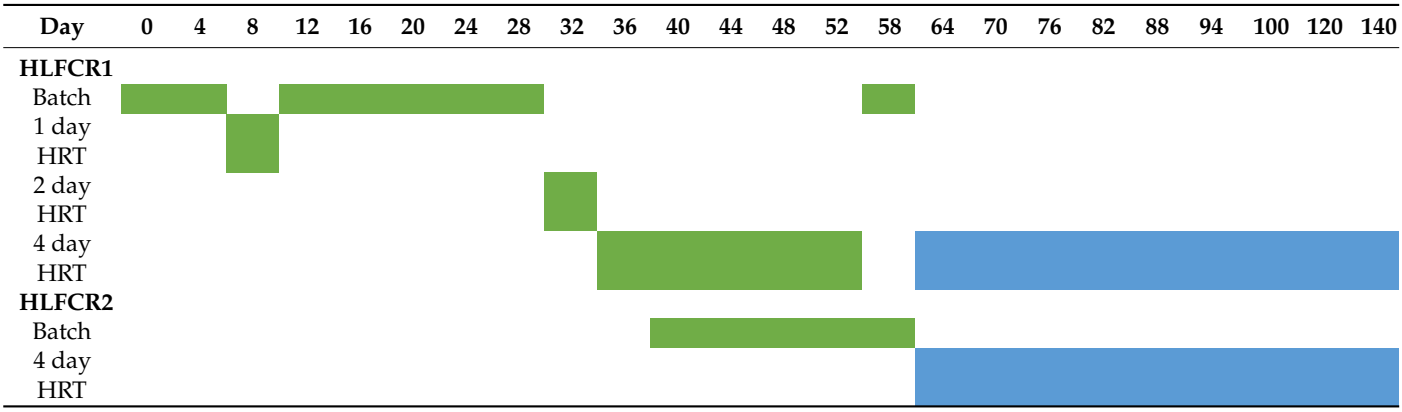
For passive volume control, the feed (influent) and effluent ports were set at the same level, resulting in a working volume of approximately 2 L based on the height. Six threaded pillars were embedded in the reactor walls, at the corners and midway along the long side, allowing a lid with an airtight seal to be fitted. The reactor contained carbon microfibers held between two aluminum plates and suspended in the bulk liquid as a surface for microbial attachment to prevent microbial washout (Figure 1A). The front of the reactor contained three sampling ports at approximately half the height of the bulk liquid (Figure 1A,B). Each port was sealed with a rubber septum allowing sampling using a syringe fitted with a 70-mm hypodermic needle. A plastic mesh screen was used to harvest the biofilm. This was suspended on wire supports just below the liquid surface. A rectangular weir was fitted to the outlet port to prevent washout of the biofilm.

Two additional ports, located midway between the influent/effluent ports and the lid, allowed air flow and could be opened or closed depending on the O_2 requirements.

Two HLFCRs were employed (HLFCR1 and HLFCR2). HLFCR1 was started with raw TWW (Section 2.2) and maintained in batch mode for six days until a thick floating sulfur biofilm (FSB) completely covered the reactor surface. Due to the variability in the composition of the TWW batches, it was necessary to blend raw and partially treated TWW to achieve a feed with relatively consistent HS^- (350 mg/L) and SO_4^{2-} (2500 mg/L) concentrations. Continuous operation was started at a 1 day HRT, but it quickly became apparent that the feed rate was too high as the residual SO_4^{2-} concentration rapidly increased. Batch mode was thus employed until almost all the SO_4^{2-} in the bulk liquid was reduced (day 31). The system was changed back to continuous mode at a 2-day HRT for one cycle; after which, the feed rate was further reduced to achieve a 4 day HRT, which was maintained for the duration of the experiment (104 days). The SO_4^{2-} concentration measured in the effluent remained above the desirable range, so it was decided to evaluate two reactors in the series.

On day 41, HLFCR2 was started with 50% (*vol./vol.*) mixed enriched cultures (Section 2.1) and raw TWW (Section 2.2) and operated in batch mode for 26 days. It was then connected in the series to HLFCR1. Biofilm development was monitored visually. The Gantt chart in Table 2 summarizes the process operations. The timeline is an indication and not an exact representation.

Table 2. Gantt chart describing the operational methods of HLFCR1 and HLFCR2. The green borderless blocks indicate when the reactors were run separately, blue double-bordered blocks indicate when the reactors were run in the series.



2.4. Batch Anaerobic Digestion

Batch AD tests were performed using TWW before and after pretreatment in HLCFRs. Each 1 L glass screw cap bottle reactor was fitted with a modified lid with air-tight ports for collecting biogas and for sampling reactor contents. Each reactor was inoculated with 200 mL of active AD sludge from an industrial scale facility processing abattoir effluent, 793 mL of either the TWW or diluted TWW (50% *vol./vol.*), 6 mL of macro-nutrient solution and 0.6 mL of trace element solution [19]. Positive controls containing either acetate or microcrystalline cellulose (MCC) were included (3 g COD/L). The reactors were sparged with nitrogen gas (N_2), sealed and placed on a rotary shaker (120 rpm) in an incubator at 38 °C. The reactors were sampled periodically, based on gas production, over 14 days.

2.5. Sampling

2.5.1. Hybrid Linear Flow Channel Reactors

Daily samples (5 mL) were drawn from the first (front) and third (back) sample ports using sterile hypodermic needles, and effluent samples were collected from the effluent ports.

The sulfur biofilm was harvested by removing the mesh screen and scraping off the accumulated biofilm. The material was dried at 70 °C for 48 h, then weighed.

2.5.2. Anaerobic Digesters

The AD reactors were sampled by withdrawing 5 mL using sterile syringes. Biogas was collected in gas sampling bags connected to the ports in the lids of the reactors.

2.6. Analytical Methods

The HS^- concentrations in the HLFCR samples were analyzed immediately on sampling, followed immediately by determination of the pH and redox potential. Thereafter, 2 mL was centrifuged (14,000 rpm for 5 min), and the concentrations of SO_4^{2-} total and soluble COD were determined in the supernatant fluid. The pH of the samples from the AD reactors was determined immediately. The samples were then centrifuged at 14,000 rpm for 5 min and the supernatant filtered through a 0.45-µm filter before being used to measure soluble COD and NH_4^+ concentrations.

The pH, electrical conductivity and redox potential were measured using a Eutech PC2700 m fitted with the appropriate probes.

The S^{2-} and SO_4^{2-} concentrations were determined spectrophotometrically using the N,N-dimethyl-p-phenylenediamine (DMPD) and barium chloride (BaCl) techniques, respectively [20]. Merck Spectroquant® test reagents and kits were used to quantify the COD (cat no: 1.14538.0065, 1.14680.0495, 1.14679.0495 and 1.14680.0495) and TAN (cat no: 1.14752.0001 and 1.00683.0001) according to the manufacturer's instructions.

The elemental composition (C, H, N and S) of the FSB was determined at the Central Analytical Facility (CAF) at Stellenbosch University (Stellenbosch, South Africa) using an Elementar (Hamburg, Germany) Vario EL cube Elemental analyzer according to the manufacturers' instructions.

The biogas composition was measured using a Geotech BIOGAS 5000 portable gas analyzer and the volume determined by displacement using an inverted, graduated 1 L measuring cylinder. The cylinder was filled with acidified water to prevent CO_2 dissolution.

3. Results and Discussion

3.1. Operation and Performance of Hybrid Linear Channel Reactors for Removal of Sulfur Species

The tanning and wastewater treatment processes used in the study tannery cannot be divulged due to proprietary and confidentially reasons. The initial "proof of concept" evaluation was performed using HLFCR1 with the assumption that the partially treated TWW would contain endogenous SRBs from existing bioreactors.

A thin FSB formed on the surface of the reactor within the first 24 h after start-up, and a week later, there was a noticeable reduction in the odor. At this point, the FSB was disrupted, and the reactor switched to continuous mode with a 1 day HRT. This proved too ambitious, and the high flowrate resulted in a drop in HS^- and increase in SO_4^{2-} concentrations in the bulk liquid. The reactor was switched back to batch mode until almost all the SO_4^{2-} had been reduced; after which, continuous feeding was restarted, initially at a 2 day HRT, but ultimately, a 4 day HRT was where the most stable performance was achieved. The pH in the bulk liquid remained relatively stable for the duration of the experiment due to the high alkalinity of the TWW and additional bicarbonate (HCO_3^-) generated during BSR (Equation (1)). The average pH was around pH 7.5, meaning that the majority (70%) of the S^{2-} in the system would be present as HS^- rather than the more toxic H_2S. The pH of the effluent was consistently higher (0.6 pH units) than the bulk liquid in HLFCR1, which was assumed to be due to the partial oxidation of HS^- to S^0, releasing OH^- ions (Equation (2)). Similarly, the redox potential in the bulk liquid remained below −380 mV for the duration of the run, indicating that the anaerobic conditions needed for BSR were present in the bulk liquid.

The HS^- and SO_4^{2-} concentrations measured immediately after HLFCR1 was started were 187 mg/L and 3200 mg/L, respectively (Figure 2A,B). The HS^- concentration de-

creased rapidly over the first few days when the FSB was absent or very thin, which allowed O_2 to readily enter the bulk liquid. Complete FSB formation is crucial to the reactor's operation, so that O_2 mass transfer to the bulk liquid is limited, and BSR can occur in an anaerobic environment.

After the initial attempt to run continuously (days 7–9), HLFCR1 was run in batch mode for 21 days until it achieved near-complete SR. The mean SO_4^{2-} concentration in the bulk liquid decreased from 2923 mg/L to 41 mg/L during this period (Figure 2B). The measured HS^- was <144 mg/L (Figure 2A), despite near-complete SR having taken place. This suggested that the majority of the HS^- generated was partially oxidized, either in the developing FSB or to form colloidal S^0 in the bulk liquid. By the end of the batch phase, the FSB was complete and thick. However, when continuous feeding was started, initially at a 2 day HRT, there was some fragmentation and washout of FSB, particularly from the edges. By day 40 at a 4 day HRT, the system attained a degree of stability, showing an increase in the HS^- concentration and a relatively consistent SO_4^{2-} concentration (Figure 2). At this point, the FSB was harvested. By day 46, thick FSB covered 80% of the liquid surface in HLFCR1. The presence of an intact FSB resulted in a steady decrease in the SO_4^{2-} concentration and accompanying increase in HS^- in the bulk liquid. The S^{2-} concentration measured in the effluent was consistently close to 0 mg/L between day 30 and 42, indicating near-complete SO.

During the development of the HLFCR system using synthetic mine impacted water, Marais et al. [16] observed a significant decrease in the S^{2-} concentration in the bulk volume in the 24 h following FSB disruption or harvesting due to unimpeded O_2 mass transfer, leading to SO in the bulk volume. The authors found that, at lower S^{2-} concentrations (<40 mg/L), SR was inhibited, leading to catastrophic reactor failure. In this study, the high S^{2-} in the TWW mitigated against a more significant decrease in HS^- concentration within the reactor and helped ensure that the bulk volume remained anaerobic, even when the biofilm was not complete, a notable finding.

One of the challenges experienced with operation of the HLFCR1 was the periodic accumulation of S^0 or sludge at the outflow point. This prevented flow from the reactor and caused an increase in volume until the pressure forced the solids out and the accumulated volume drained from the reactor. This accounted for an anomalous increase in the S^{2-} concentration in the effluent between days 43 and 50 (Figure 2).

The second HLFCR (HLFCR2) was connected to HLFCR1 in the series on day 63, so it was no longer possible to sample the effluent from HLFCR1. The effluent results given in Figure 2 from day 63 were therefore the final results from the reactors in the series (effluent from HLFCR2). Nevertheless, the results obtained from the analyses of the bulk liquid in HLFCR1 indicated that BSR continued to improve, and the S^{2-} concentration reached a maximum of 879 mg/L on day 87 (Figure 2A). During this period, the pH was 7.7, so approximately 80% of the S^{2-} would have been present as HS^-, resulting in a H_2S(aq) concentration of 186 mg/L, which theoretically should not have inhibited an acclimated SRB community [21,22].

Following the disruption of FSB on day 97, there was a period of 3 to 4 days while it reformed. During this period, the residual SO_4^{2-} concentration increased, and the HS^- decreased. Biofilm regeneration took longer in this system compared to the model system that was operated with simple, defined growth media [16]. The FSB also appeared to have less structural integrity. This may have been related to the impact of the high salinity on the composition of the heterotrophic bacterial community thought to be responsible for the organic scaffold of the FSB.

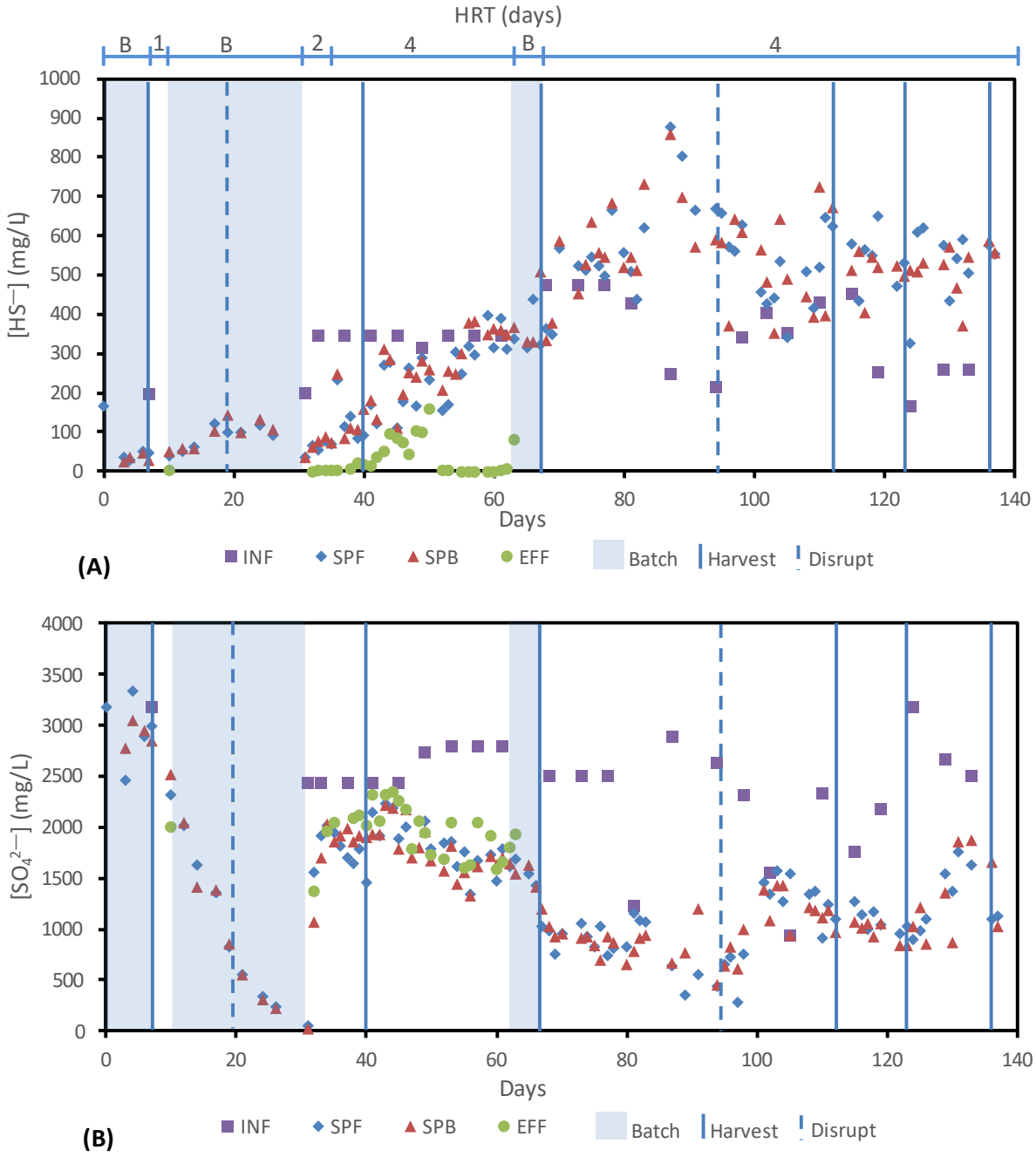


Figure 2. Change in the (**A**) HS^- and (**B**) SO_4^{2-} concentrations in HLFCR1. Vertical lines and shading indicate biofilm harvest and disruption events and periods of batch operation. INF = influent, SPF = sampling port front, SPB = sampling port back and EFF = effluent.

From day 101 to the end of the experiment, the system operated with fewer disruptions. The HS^- concentration in the bulk liquid remained relatively stable around 520 mg/L, with the SO_4^{2-} concentration at approximately 1000 mg/L. This equated to an average conversion efficiency of 56% of the feed SO_4^{2-} between days 101 and 138. The results compare favorably with those obtained by Boshoff et al. [23], who used anaerobic reactors dedicated to the BSR of TWW. The authors achieved a maximum removal of 1.08–1.44 g SO_4^{2-}/L at a 4 day HRT in an upflow anaerobic sludge blanket reactor (UASB), albeit at a higher efficiency (60–80%). In this study, at the same HRT (4-day), an average removal of 1.23 g SO_4^{2-}/L was achieved in HLFCR1, but in this case, the BSR was accompanied by SO. Studies investigating both SO_4^{2-} and HS^- removal [16,24] have also

achieved good SO_4^{2-} removal by using defined media supplemented with an electron donor (lactate). Xu et al. [24] achieved 81.5% removal (0.82 g SO_4^{2-}/L at a 1 day HRT), while Marais et al. [16] achieved 96% removal (0.96 g SO_4^{2-}/L) at a 4 day HRT. Given the fact that this study was conducted using a complex saline effluent (TWW), the results are promising and provide concrete evidence that HLFCRs may be useful for the pretreatment of TWW for the removal of S species.

While the performance of HLFCR1 proved that HLFCRs could be used for the removal of S species from TWW, the residual SO_4^{2-} concentrations in the effluent were still above the desired level. Therefore, HLFCR1 and HLFCR2 were operated in the series for the remainder of the experimental period. Unlike HLRCR1, HLFCR2 was inoculated with exogenous cultures, as described in the Materials and Methods.

The data indicated a period of adaptation in HLFCR2 during the start of the batch phase, during which time the HS^- concentration decreased from 534 mg/L on day 43 to a minimum of 84 mg/L on day 56 (Figure 3A). This was not due to an increased SO but, rather, a lack of SR (Figure 3B).

The HS^- concentration in the bulk liquid increased further after HLFCR2 was switched to a continuous operation, reaching a maximum of 658 mg/L on day 87. The average S^{2-} concentration was typically 80–100 mg/L lower than in the bulk volume of HLFCR1, possibly due to the lower concentration of SO_4^{2-} in the influent (effluent from HLFCR1). The S^{2-} concentration in the effluent from HLFCR2 was consistently <5 mg/L during stable operations. As with HLFCR1, there was an occasional accumulation of solids at the effluent port, accounting for anomalous spikes of S^{2-} in the effluent. The redox potential decreased from about −475 mV in the influent to -369 ± 83 mV in the effluent from HLFCR2.

The minimum bulk liquid SO_4^{2-} concentration (109 mg/L) was measured on day 76 and represented an excellent SR efficiency of 96.6% for the system in the series. The biofilm was then disrupted, which was followed by a period of increase in SO_4^{2-} until day 104; after which, it remained relatively stable at approximately 800 mg/L. During this latter period of stable operation, it was possible to harvest the FSB more frequently. As with the effluent S^{2-} spikes, the variability in the effluent SO_4^{2-} concentration was primarily due to the imperfect design of the effluent port. In some cases, there was accumulation of a small volume in the weir, where some complete sulfide oxidation occurred, inflating the measured sulfate value. Overall, the system achieved an average SR efficiency of 65–70%, with an average influent concentration of 2500 mg/L and near-complete removal of S^{2-}.

3.2. Sulfur Species Removal Rates

The SR rate (SRR) and SO rate (SOR) data for each HRT are plotted in Figure 4. HLFCR1 was able to achieve SRRs of up to 1049 mg SO_4^{2-}/L.day (10.9 mmoles/L.day) in the bulk liquid, while HLCFR2 reached 513 mg SO_4^{2-}/L.day (5.3 mmoles/L.day), with an overall rate of 444 mg/L.day (4.6 mmoles/L.day). The higher SO_4^{2-} loading and utilization of the more readily available substrates in the influent to HLFCR1 were responsible for higher rates being achieved in HLFCR1 than in HLFCR2. These SRR were on par with those obtained by Boshoff et al. [23], where TWW was treated using active methods, achieving 250–600 mg SO_4^{2-}/L.day. Studies that achieved higher rates (e.g., 2200 and 2920 mg/L.day by Oyekola et al. [4] and Sabumon [25], respectively) were most likely due to higher SO_4^{2-} loading (10 and 2.85 g/L.day, respectively). In addition, these studies used more readily available carbon sources in comparison to TWW. Loading directly affects the SRR, with a near-first-order relationship until substrate inhibition occurs [26].

Figure 4A,B indicates when the influent TWW batches were changed and which batches of raw and partially treated TWW were used to prepare the blends. While the changes in the influent did not seem to have a consistent impact on the SRR, it did appear that the SRR decreased as the influent (feed) aged, which was assumed to be due to microbial activity taking place in the feed bottles over the 4 day HRT.

The SOR was more inconsistent than the SRR (Figure 4B). The SOR was calculated based on the difference in HS^- concentration between the bulk liquid and effluent initially

in HLFCR1 alone (HRT 1–8) and, then, from the effluent of HLFCR2 (HRT 9–18). From HRT 19 until the end of the experiment (HRT 23), an "effluent" sample was also taken from the weir of HLFCR1. Initially, the HS^- concentration in the bulk liquid of HLFCR1 was relatively low (Figure 3A), so even with near-complete HS^- oxidation, the SOR was below 150 mg/L.day (4.6 mmoles/L.day). The HS^- measured in the bulk volume of HLFCR2 was higher, resulting in an increase in the SOR. The fact that the HS^- concentration measured in the effluent from HLFCR2 was typically <5 mg/L indicates that the SOB community was metabolically active and that the SOR measured was dependent on the amount of HS^- present largely as a result of SRB activity rather than being constrained by inhibition.

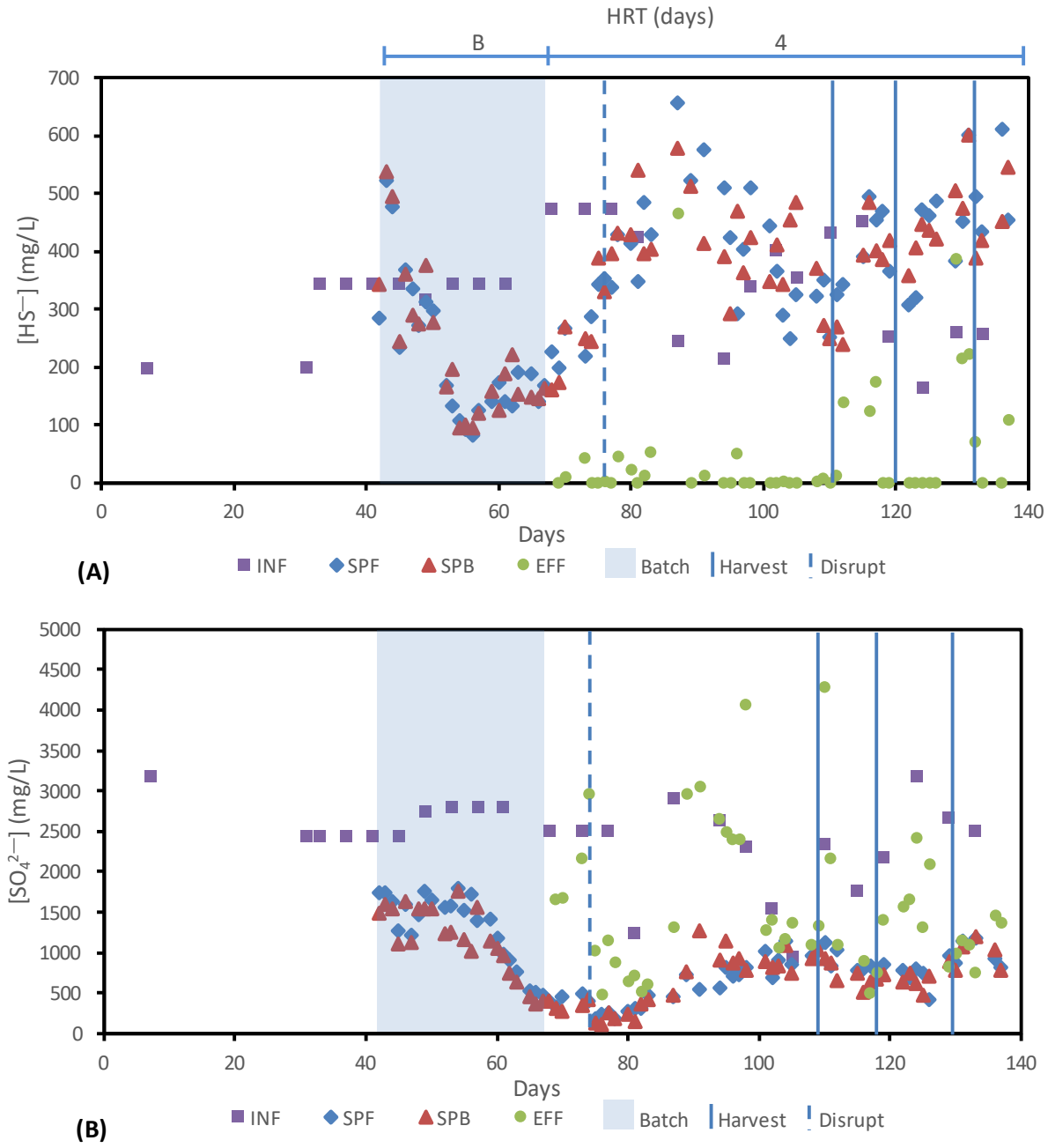


Figure 3. Changes in the (**A**) HS^- and (**B**) SO_4^{2-} concentrations in HLFCR2. Vertical lines and shading indicate the biofilm harvest and disruption events and periods of batch operation. INF = influent to HLFCR1, SPF = sampling port front, SPB = sampling port back and EFF = effluent.

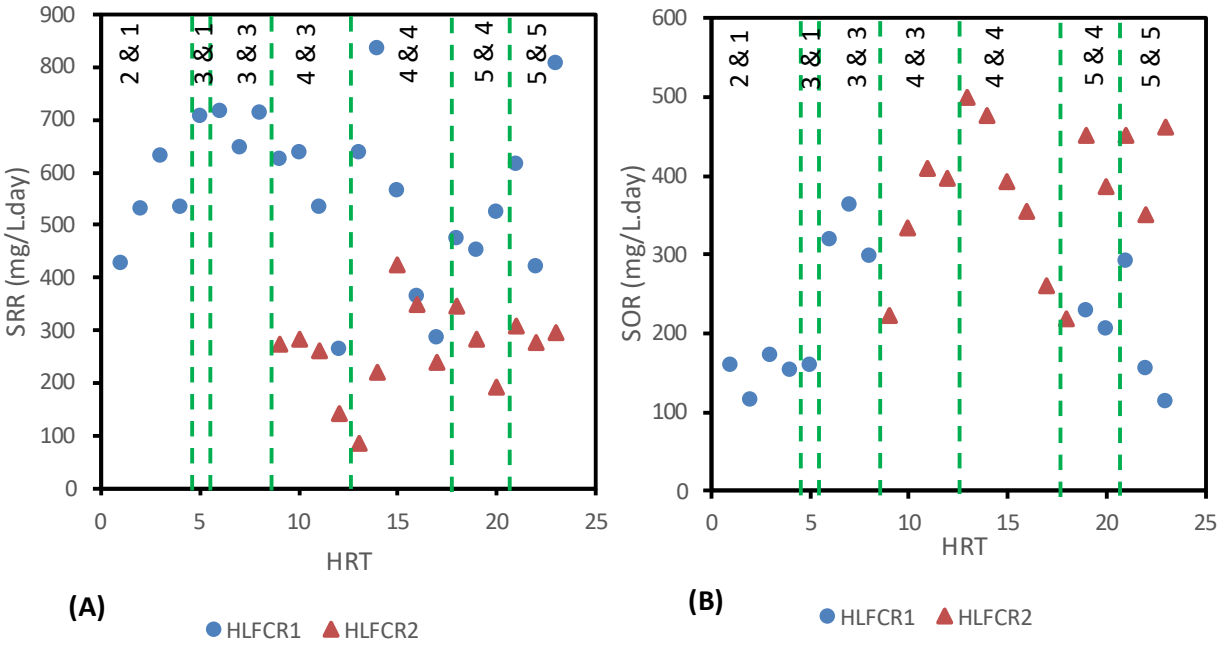


Figure 4. Average sulfate reduction rates (**A**) and sulfide oxidation rates (**B**) in the hybrid linear flow channel reactors across each subsequent 4-day hydraulic retention time.

Overall, the system achieved an average SOR of 322 mg/L.day (10 mmoles/L.day), which compared favorably to the rates achieved by Mooruth [17] in an LFCR (maximum of 184 mg/L.day) and Xu et al. [24] in a micro-aerophilic expanded granular sludge bed (EGSB) reactor with lactate as the carbon source (183 mg/L.day). The latter study identified SO as the rate-limiting step in their process, which was not the case for this study. The overall SOR was higher on a molar basis than the SRR. The presence of HS^- in the TWW feed meant there was more HS^- available for oxidation than was produced by the bacterial sulfate reduction, so the SOR could exceed the SRR.

Vertical lines indicate changes in the feed composition, with the numbers indicating the batch number of raw and partially treated tannery wastewater blended to make up the feed (Figure 4).

3.3. Floating Sulfur Biofilm Formation and Sulfur Recovery

Rapid FSB formation occurred in HLFCR1, completely covering the surface within 48 h after start-up (Figure 5A). In HLFCR2, the initial FSB formation was slower and required seeding with dried FSB from HLFCR1 to ensure complete formation within 2 to 3 days (Figure 5B). During stable operations of the reactors in the series from day 108, the FSB harvesting was staggered so that there was always a complete biofilm on the surface of the bulk liquid in one of the HLFCRs. During harvesting, some residual FSB fragments were left on the surface (Figure 5D), as this was shown to accelerate reformation.

The harvested FSB had a yellow-cream color characteristic of a S^0 content. The elemental analysis for C, H, N and S showed that the dried FSB from HLFCR1 contained less S and more C (38–58% and 7.8–11%, respectively) than that from HLFCR2 (62–77% and 6.1–6.2%, respectively). This was consistent with an expected higher proportion of heterotrophic bacteria in HLFCR1, which was, in turn, responsible for a more rapid FSB formation.

A mass balance was performed using the SO_4^{2-} and HS^- in the influent and effluent and the S recovered in the FSB. This showed that 42% of the S fed to HLFCR1 and 39% of the S fed to HLFCR2 could not be accounted for. This was primarily attributed to the presence of colloidal S in the bulk volume, which formed during periods when the FSB was incomplete after disruption or harvesting. At these times, the effluent had a milky appearance. Occasionally, the effluent samples were pale yellow–green in color, which

indicated the presence of a small amount of polysulfide (S_n^{2-}). Kleinjan et al. [27] found that the presence of S_n^{2-} accelerated the dissolution of S^0, so this was undesirable.

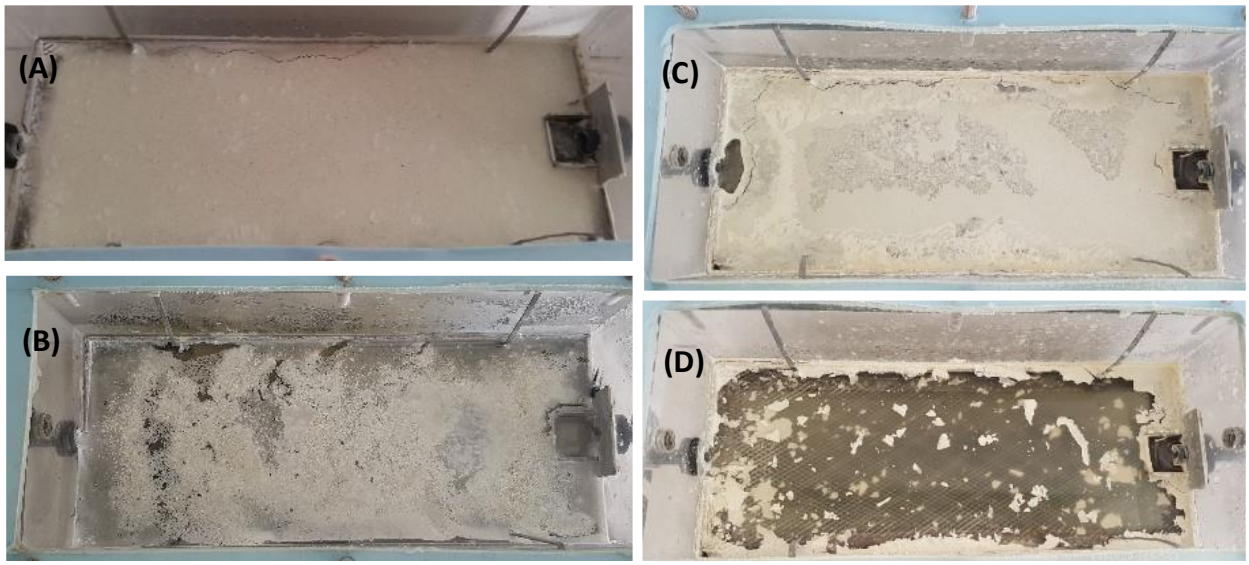


Figure 5. Floating sulfur biofilm formation. (**A**) HLFCR1, day 2, showing the first biofilm that formed. (**B**) HLFCR2, day 53, four days after seeding with saline-adapted, dried biofilm. (**C**) HLFCR1, day 40, prior to harvesting. (**D**) HLFCR1, day 40 after harvest, showing residual biofilm.

For the reasons alluded to above, S recovery from the FSB (average 6.9% and 10.2% in HLFCR1 and HLFCR2, respectively) was lower than the S recovery achieved by Mooruth [17] (92%) and Marais et al. [16] (30%) using synthetic, low-salinity media, where the majority of S was recovered in the FSB, and there was limited colloidal S in the bulk volume. It is likely that S recovery could be improved by promoting rapid FSB formation.

The current hypothesis is that FSB formation may be retarded if the heterotrophic species responsible for forming the organic scaffold that gives the FSB its structural integrity are inhibited or there is a deficiency of simple organic molecules that have been shown to stimulate scaffold formation [17]. A more detailed study of these heterotrophic organisms and their interactions with the SOBs in FSB formation during the pretreatment of TWW should provide information that will help to improve the rate of FSB formation and increase S^0 recovery.

3.4. COD Utilization

The desired outcome of the proposed pretreatment is to decrease the amount of S species while maintaining sufficient residual COD for the effluent to remain attractive as a substrate for AD. The soluble COD concentration of the influent ranged between 7 and 10 g/L, with the total COD values between 17 and 27 g/L, depending on the amount of suspended solids in each batch of TWW. The majority of the reduction in soluble COD took place in HLFCR1 (29%) compared to HLFCR2 (15%), which is consistent with the majority of the SO_4^{2-} being present in HLFCR1. The average soluble COD concentration in the final effluent was 5.3 g/L (44% COD reduction), meaning that more than half the initial soluble COD was still available for AD.

Due to the large range of possible reactions, including (i) the potential for the liberation of organics by sludge hydrolysis, (ii) the concurrent utilization of organics by SRBs, as well as by non-SRBs (fermentative microbes), in the bulk liquid and (iii) the utilization of organics by aerobic and microaerophilic microbes within the FSB, it was not possible to conduct a mass balance for COD in order to derive a definitive relationship between the amount of SO_4^{2-} reduced and soluble COD consumed. To quantify how much COD

was used during BSR and whether the amount of soluble COD being formed by sludge hydrolysis was significant, Equation (4) was applied [28]:

$$127.8\ \text{g COD} + 192\ \text{g } SO_4^{2-} + 55.8\ \text{g } H_2O \rightarrow 68\ \text{g } H_2S + 2.4\ \text{g sludge} + 244\ \text{g } HCO_3^- \quad (4)$$

The soluble COD in each reactor was measured once per HRT. The concentration data were multiplied by the reactor volume to determine the mass of soluble COD and S species. The analysis showed that HLFCR1 utilized notably more soluble COD than was required for BSR over each HRT, while the soluble COD utilization across the HLFCRs in series was almost double what could be attributed to the SR (results not shown). Okabe et al. [29] found that some S-metabolizing bacteria require relatively more energy to overcome their inhibition due to toxic levels of HS^- than is theoretically required for cell maintenance. Therefore, the surplus soluble COD usage was attributed to one or more of the following factors: (i) undiscernible BSR, where complete the SO of HS^- to $SO_4{}^{2-}$ occurred at the surface while the FSB was incomplete, (ii) the surplus energy required in overcoming HS^- inhibition and/or (iii) the energy required by other microbial community members, such as biofilm-forming heterotrophs.

3.5. Batch Anaerobic Digestion of Tannery Effluent before and after Pretreatment in Hybrid Linear Channel Reactors

It has been shown that solid tannery waste and TWW can be successfully digested if a well-acclimated inoculum is used and the initial S^{2-} and $SO_4{}^{2-}$ concentrations are kept below certain thresholds [2,9,30]. Higher initial $SO_4{}^{2-}$ concentrations that are converted to more toxic S^{2-} species by BSR during AD appear to affect the methanogenic function more than the community composition [8]. Mpofu et al. [18] co-digested TWW with slaughterhouse wastewater and achieved specific CH_4 yields of up to 146 mL/g volatile solids provided the initial $SO_4{}^{2-}$ concentration was $\leq$1960 mg/L. In this study, the high initial S^{2-} concentrations in the TWW (Table 1) made successful AD theoretically implausible. Indeed, the batch AD tests conducted on both the raw and partially treated TWW showed a complete lack of biogas generation (including CH_4, CO_2 and all the other forms of biogas). Complete inhibition was also noted with diluted TWW (50% *vol./vol.*), even with acetate supplementation (results not shown). The complete lack of any biogas-generating metabolic activity reflected the inhibition of multiple microbial groups, not only the more sensitive methanogens. In these experiments, the acetate and microcrystalline cellulose (MCC) controls were positive, validating the presence of functional microbial communities in the inoculum. Methane generation from acetate provides an indication of the methanogenic activity [30], while CH_4 generation from MCC indicates that hydrolytic and acetogenic microbial communities are also active [31]. It is possible that other factors such as salinity played a role in methanogenic inhibition, as the inoculum had not been pre-acclimated to the TWW. However, when batch AD tests were performed on the effluent from the partially treated effluent, methanogenic activity was found (Table 3).

The specific CH_4 yield, as well as the %CH_4 in the biogas, was improved by diluting the pretreated TWW. Logically, by diluting the pretreated TWW, the concentrations of all the potential inhibitors in the pretreated TWW decreased. This included a reduction in salinity. The commercial-scale digester from which the inoculum was obtained operated at an average EC value of 8.35 mS/cm, while the undiluted TWW had an EC value >30 mS/cm. The impact of the increased salinity on the methanogenic community was confirmed by the results of the acetate controls. The specific CH_4 yield in the high saline reactor was >40% lower than the low salinity control (Table 2). Similar results were found by Liu et al. [32], who showed a reduction in methanogenic activity of 20% and 57% at 5 and 10 g/L of Na^+, respectively. Supplementation of the diluted, pretreated TWW with additional acetate increased the specific CH_4 yield, as well as the %CH_4 in the biogas, suggesting incomplete acidogenesis and acetogenesis of the available organic fraction.

While it is recognized that these AD results could be improved by optimizing the selected parameters and using a well-acclimated inoculum, the intention of the AD experi-

ments was to show that the AD of TWW could be improved by pretreatment in HLFCRs. This hypothesis was well-validated by the AD results obtained.

Table 3. Methane generation from pretreated tannery wastewater.

Methane Yield	Controls		Pretreated Tannery Wastewater		
	Acetate	Acetate + NaCl	Undiluted	50% Dilution	50% Dilution + Acetate
mL CH_4/g $COD_{consumed}$	342	208	38.4	130	214
% of biogas	69.7	42.45	13.7	32.5	46.8

4. Conclusions

Untreated TWW is generally not suitable for AD. This study showed that HLFCRs could be used to pretreat tannery effluents, achieving good SRR and near-complete S^{2-} removal accompanied by a 40% reduction in COD. This pretreatment could be improved by manipulating operating conditions to improve the formation of FSB, which was rate-limiting in this study. The composition of the TWW will dictate whether a single reactor or multiple reactors in a series will be necessary to achieve the desired reduction in the sulfur species. It was conclusively demonstrated that the pretreatment of TWW in HLFCRs renders the substrate more amenable to AD, although the performance could be improved by optimizing the operational conditions and utilizing a well-acclimated inoculum.

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Abbreviations

ASW = artificial seawater; HLFCR = hybrid linear flow channel reactor; TWW = tannery wastewater; SO = sulfide oxidation; SOR = sulfide oxidation rate; SR = sulfate reduction; SRR = sulfur reduction rate.

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