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Resource recovery from tannery wastewater using an integrated biological system: Towards a circular bioeconomy and net positive tannery operations

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ABSTRACT

Anaerobic digestion (AD) of tannery effluents generated by beamhouse processes is limited by ammonia and sulfur species. High sulfate concentrations in beamhouse effluents (BHE) promote sulfidogenesis during AD, and the hydrogen sulfide that is generated inhibits methanogenesis. The feasibility of using a novel integrated biological system that comprised of hybrid linear flow channel reactor/s (HLFCR/s) and an anaerobic sequential batch reactor (AnSBR) connected in series mainly for the pre-treatment and recovery of elemental sulfur (S^0) and methane (CH_4) was investigated. The application of single-stage and two-stage HLFCR topology operating at 8 and 4 days' hydraulic retention times (HRT), respectively, produced effluents that were ideal for AD. Single-stage HLFCR (8-days HRT) recovered 21% of the inlet sulfur and the downstream effluent treatment in continuously mixed AnSBR at 50 rpm achieved 238 mL $CH_4/gCOD_{added}$). The full-scale application of the system at a local medium sized tannery treating 2258 m^3 of BHE would produce a floating sulfur biofilm with 33% S^0, 3420 m^3 of CH_4, 50 m^3 of irrigation water and 31 tonnes of biofertiliser. The sale of recovered resources and potential savings from a 72% reduction in electricity demand and 62% in sludge disposal had a potential revenue of US $5559. This study demonstrated the feasibility of a circular bioeconomy and net positive tannery operations through the conversion of tannery wastewater treatment plants into bio-refineries.

1. Introduction

Tanneries are an economically important agri-processing subsector in many developing countries. Tanneries alleviate the potential environmental burden of disposing skins/hides generated by the slaughterhouse industry, but create another by generating large volumes of highly toxic effluents, particularly from beamhouse (BH) processes. Generally, BH processes produce 20–25 m^3 of effluent per tonne of skins/hides processed. The beamhouse effluent (BHE) contributes about 79% suspended solids, 75% of biological and chemical oxygen demand (BOD and COD), 74% chlorides (Cl), 100% sulfide (HS^-/S^{2-}), and 85% total nitrogen (TN) to the total pollution load (Buljan and Král, 2019). The typical concentration ranges of dominant BHE pollutants: SO_4^{2-} (270–2400 mg/L), total sulfide (250–525 mg/L), ammonia/um (96–865 mg/L), and Cl (900–9025 mg/L) (Mpofu et al., 2022; Wiemann et al., 1998). The widely used activated sludge process coupled with physicochemical treatments is not effective in treating BHE to stipulated South African discharge standards, particularly for sulfate (SO_4), sodium (Na), chloride (Cl) and organics (typically measured as COD) (Mpofu et al., 2021; Swartz et al., 2017), resulting in penalties upon discharge.

The work by Mpofu et al. (2022), Mpofu et al. (2021) and Mpofu et al. (2021b) reported on the feasibility of using anaerobic digestion (AD) for the recovery of methane (CH_4), biofertiliser and process/irrigation water treatment from tannery wastewater (TWW). The authors reported on the concurrent occurrence of methanogenesis and sulfidogenesis at low COD: SO_4^{2-} ratios (<6.3) resulting in increased sulfide concentrations that fell within the inhibitory range (IC_{50} = 43–125 mg/L at pH 7–8) for functional methanogenic archaea (Kibangou et al., 2021; O'Flaherty et al., 1998). The authors observed the formation of elementary sulfur (S^0) at the air-liquid interface of the reactors. It was postulated that a small fraction of the sulfides were partially oxidised by nitrite (NO_2^-) and nitrate (NO_3^-) into S^0 by chemolithotrophic sulfur oxidising bacteria (SOB) and anaerobic ammonium oxidation (ANAMMOX) (Mpofu et al., 2022). However, maximum

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CH_4 and S^0 recovery could not be achieved due to inhibition by SO_4^{2-}/H_2S, ammonia (NH_3), volatile organic acids (VOAs) and oxidising agents (Kibangou et al., 2021; Mpofu et al., 2022). The authors recommended a pre-treatment step for the removal and/or recovery of these inhibitors, particularly sulfur species in order to make BHE more amenable for AD (Mpofu et al., 2021b, 2022).

Aqueous sulfides (HS^-/H_2S) are commonly removed from BHE through precipitation and/or oxidation, by adding coagulants, oxidising catalysts and/or extended aeration (Swartz et al., 2017). A number of studies have successfully applied coagulation ($FeCl_3$, $Ca(OH)_2$, and $Al_2(SO_4)_3$, stripping and hydrolysis as pre-treatment steps for the removal/recovery of sulfides and Cr prior to AD of TWW (Aboulhassan et al., 2008; Song et al., 2001; Song and Williams, 2004). However, pre-treatment sometimes leads to decreased biogas/ CH_4 yields and/or anaerobic biodegradability (B_0) due to increased concentrations of inhibitory cations (Fe, Ca and Al) and/or anions (SO_4^{2-}, Cl^- and OH^-), and precipitation of organic matter, and nutrients (Genschow et al., 1997; Schenk et al., 1999; Wiemann et al., 1998). These pre-treatment approaches are also expensive due to the associated chemical and energy costs. In addition, the disposal costs of excess sludge laded with coagulants further increases the cost (Buljan and Král, 2019; Mpofu, 2018). Attempts by tanners to eliminate sulfur based feed chemicals such as chrome sulfate ($Cr_3(SO_4)_3$) and sodium sulfide (Na_2S) have not been successful due to increased capital costs and perception of compromising the quality of leather (Mpofu et al., 2021). Therefore, there is a need for efficient and cost effective alternative methods for removing sulfur species ($SO_4^{2-}/HS^-/H_2S$) in TWW to promote AD.

Biological SO_4^{2-} reduction (BSR) is potentially a cost-effective alternative pre-treatment method for removing/recovering sulfur and nitrogen species from TWW (Sabumon, 2008a, 2008b). The majority of biological processes for $SO_4^{2-}/HS^-/H_2S$ removal employ two-stage treatment for BSR and sulfide oxidation (SO). However, Sabumon (2008b) demonstrated the feasibility of recovering S^0 from pre-treated TWW by integrating BSR and SO in an up-flow hybrid anoxic bioreactor while injecting air from the bottom. Marais et al. (2020) employed a novel hybrid linear flow channel reactor (HLFCR) that integrated BSR and SO for the recovery of S^0 from synthetic wastewater (1 g/L SO_4). The HLFCR is a novel semi-passive reactor design that spatially separates anaerobic and aerobic zones while maintaining a very close interaction between the microbial species (Horn et al., 2022b). The hydrodynamic profile (limited turbulence) within the HLFCR creates an environment that is conducive for the formation of a harvestable floating sulfur biofilm (FSB) at the air-liquid interface and the settling of suspended solids. The FSB is crucial for creating the pH/redox environment that supports the partial oxidation of sulfides into S^0 (Equation 1 – Equation (3)). Complete sulfide and S^0 oxidation due to excess O_2 is undesirable as it produces SO_4^{2-} and acidity. The HLFCR was applied by Horn et al. (2022b) on the pre-treatment of TWW and this preliminary study demonstrated the feasibility of recovering S^0 from TWW.

This study therefore sought to build on the work presented in Horn et al. (2022), Mpofu et al. (2022), Mpofu et al. (2021) and Mpofu et al. (2021b) that focused on the biological treatment of TWW for resource recovery other than biogas. The overall aim of the study was to develop an integrated biological treatment process for the recovery of reusable/recyclable sulfur, biofertiliser, and process/irrigation water, while generating methane-rich biogas, from bovine/ovine beamhouse effluent. The process is based on an integrated HLFCR and anaerobic sequential batch reactor (AnSBR) in series and seeks to promote a circular bioeconomy and net positive tanneries in developing countries. To the authors' knowledge, this is the first time that an HLFCR coupled with an AnSBR has been optimised for the recovery of resources besides biogas from bovine/ovine beamhouse effluent.

$$\textbf{electron donor} + \mathbf{SO_4^{2-}} \rightarrow \mathbf{HS^-} + \mathbf{HCO_3^-} \quad \text{Equation 1}$$

$$\mathbf{2HS^- + O_2 \rightarrow 2\,S^0\downarrow + 2OH^-} \quad \text{Equation 2}$$

$$\mathbf{S^0 + H_2O + \frac{3}{2}O_2 \rightarrow SO_4^{2-} + 2H^+} \quad \text{Equation 3}$$

2. Materials and methods

2.1. Inoculum and substrate

The BHE used in this study was collected from a South African tannery that processes bovine hides and ovine skins via wet-blue tanning. A robust, acclimated microbial seed consortium was obtained from biomethane potential (BMP) experiments used to treat BH and tanyard (TY) effluents as described by (Mpofu et al., 2022). A saline-adapted BSR culture acclimated to BH effluent supplemented with lactate was used as an inoculum during start-up of a HLCFR.

2.2. Set-up and operation of hybrid linear flow channel reactors

The pre-treatment experiments were performed in 8.1 L (450 mm (l) x 200 mm (w) x 150 mm (h)) HLFCRs. The bioreactors (Fig. 1A) were fitted with airtight lids and carbon microfibers were suspended on aluminium plates in the bulk liquid (Horn et al., 2022). The microfibers promoted microbial biofilm attachment and prevented biomass washout. A plastic mesh screen positioned just below the bulk liquid and gas interface was used to harvest the FSB. The HLFCRs were equipped with three sampling ports at the front, at approximately half the height of the bulk liquid. Additionally, air vents on each side of the bioreactor allowed airflow in the headspace.

The HLFCRs were initially inoculated with BSR culture blended with BHE (10% v/v) and maintained in batch mode for seven days to allow for the development of an FSB at the air liquid interface. The FSB was partially harvested on day 7 and 14 by physical disruption. The HLFCRs were thereafter operated at 4-days HRT for 32 days as previously described by Horn et al. (2022). The FSB was by physical disrupted every fourth day and the bulk liquid was sampled for analysis. Thereafter, the HLFCRs were connected in series and maintained at a 4 day HRT for an additional 24 days to assess the impact of two-stage treatment on process efficiency. One HLFCR was later operated at an 8 day HRT for 16 days to ascertain the impact of increased HRT. The treated effluents from single and two-stage HLFCR was used to feed the AnSBRs.

2.3. Set-up and operation of anaerobic sequential batch bioreactors

Two 20 L gas-tight polyethylene AnSBRs were set up (Fig. 1B) and operated at an inoculum to substrate ratio (ISR) of 2.5 (VS/VS), 37 ± 2 °C, and pH 7 ± 0.5 (Mpofu et al., 2022). However, an ISR of 4 was used during the AD of settled solids from the HLFCRs (Mpofu et al., 2020b). The AnSBR contents were mixed using a Heidolph Instruments (Schwabach, Germany) Hei-torque 100 programmable overhead stirrer connected to a shaft and four blade marine impeller (Fig. 1B). The effect of mixing on the treatment of pre-treated BHE using HLFCR was investigated by mixing the reactor contents at different mixing conditions: continuous/intermittently; mixing time (0, 12, and 24 h per day) and speed (0, 50, 100 and 200 rpm). The overhead stirrer was programmed to mix intermittently or continuously at a certain speed by defining the mixing intervals corresponding to the required mixing time i.e. mixing for 6 or 12 min every 12 min which translates to mixing time of 12 and 24 h (continuous) per day, respectively. The operation of AnSBRs is described else by Kibangou et al. (2021).

2.3.1. Biogas sampling and analysis

The gas ports from the AnSBRs were connected to individual Tedler bags. The collected biogas was analysed quantitatively and qualitatively (CH_4, CO_2, O_2 and H_2S) using a graduated gas tight luer lock syringe

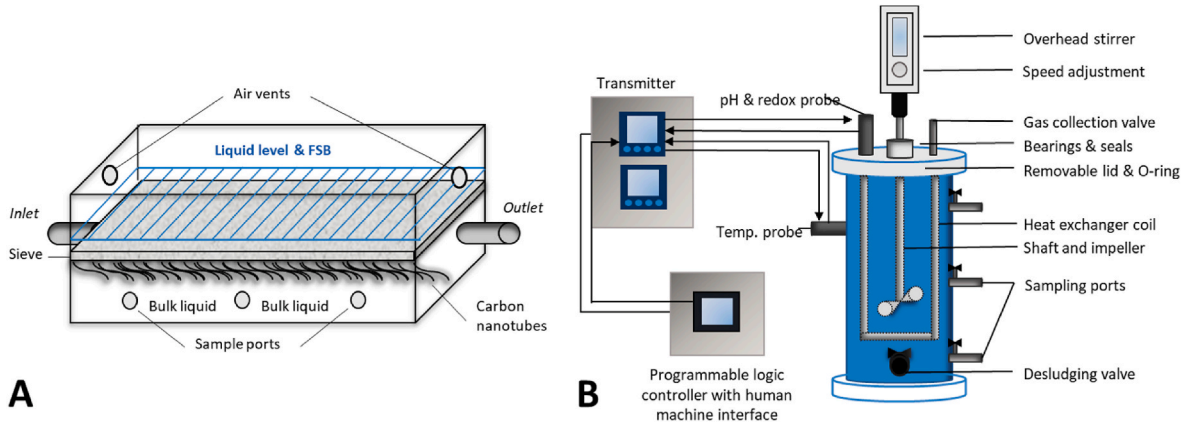

Fig. 1. Hybrid linear flow channel reactor (A) and anaerobic sequential batch reactors, and related control systems (B).

(Lasec, South Africa) and Geotech biogas 5000 analyser (Warwickshire, England), respectively.

2.3.2. Determination of physicochemical parameters

Samples for analysis were obtained at the beginning (feed) and at the end from each reactor to determine process effiiency. A Merck (Darmstadt, Germany) Spectroquant Prove® spectrophotometer together with Merck cell tests or kits were used to determine the concentrations of different parameters. Solids concentrations were determined using standard gravimetric methods at 105 °C and 550 °C for total solids and total volatile solids, respectively. Major and minor elements were quantified using a Thermo ICap 6200 ICP-AES instrument for trace analyses, and an Agilent (Santa Clara, USA) 7900 ICP-MS instrument for ultra-trace analyses. Quantification of CHNS (%wt) was done using an Elemental Vario EL cube Elemental analyser (Hamburg, Germany). Detailed information on qualitative analysis of BHE is described elsewhere Mpofu et al. (2022), and Mpofu et al. (2021).

2.3.3. Economic analysis

An economic evaluation of the integrated treatment system was conducted based on the assumption that processing a bovine hide requires about 1.9–4.4 kWh (Swartz et al., 2017), and producing 1 MWh of this electricity from coal releases 0.90 t CO_2 eq (Climate Transparency, 2021) while BHE treatment using CAS produces 3.15 t CO_2 eq/tCOD$_{removed}$ (Giaccherini et al., 2017). Electricity generation from biogas was calculated assuming an average calorific value of 6 kWh/m^3 and a conversion efficiency of 35% (Agustini et al., 2019).

3. Results and discussion

3.1. Performance of hybrid linear flow channel reactors in recovering sulfur

3.1.1. Single-stage treatment

The formation of the FSB at the air-liquid interface was observed after 24 h. This was followed by the colonisation of the carbon microfibres in the bulk liquid. After 7 days of operation, the mesh screen was fully covered by a white-cream layer of FSB (Fig. S1, supplementary). This colour resembled the physical characteristic of the expected S^0 which was partially harvested on day 7. The SO_4^{2-} concentration decreased by 78% (to 420 mg/L) after the first 7 days and by 70% (to 575 mg/L) on day 14. A similar positive trend was observed with residual ionised sulfide (HS^-) concentration which increased from 456 mg/L on day 7–594 mg/L on day 14 (Fig. 2). After day 14 (4-day HRT), the residual SO_4^{2-} and S^{2-} concentrations ranged between 853 and 1200 mg/L (38–56% reduction) and 358–471 mg/L (50–63% reduction) respectively, for the rest of the study (Fig. 2). This translated to residual average concentrations of 907 mg SO_4^{2-}/L (45% reduction) and 461 mg S^{2-}/L (28% reduction). Horn et al. (2022) operating an HLFCR at 4-day HRT made similar observations with residual SO_4^{2-} and S^{2-} concentrations that stabilised at around 1000 and 520 mg/L in the bulk liquid, respectively. The authors reported an overall BSR of 1230 mg SO_4^{2-}/L and SO rate of <150 mg S^{2-}/L.day using single-stage treatment HLFCR at 4-day HRT. These results were comparable to those achieved by

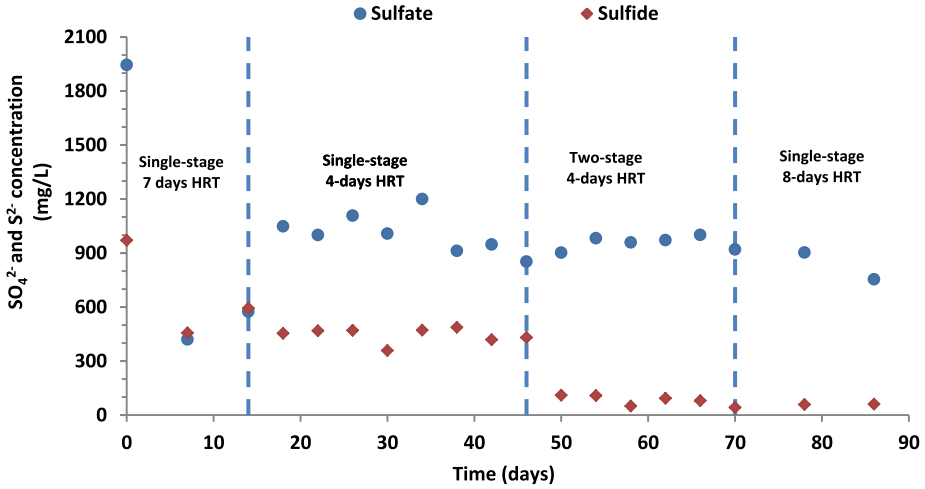

Fig. 2. Changes in the final S^{2-} and SO_4^{2-} concentrations in hybrid linear flow channel reactors operating at different hydraulic retention times and topology.

previous studies (Boshoff et al., 2004; Horn et al., 2022; Huang and Khanal, 2004; Marais et al., 2020; Sabumon, 2008a, 2008b; Xu et al., 2012).

Notably, SO_4^{2-} and S^{2-} removal was lowest at 4-day HRT and higher at 7 days HRT (Fig. 2). The observed trend may have been as a result of impeded oxygen (O_2) mass transfer across the liquid surface after the formation of FSB over 7 days. Sulfide oxidation (SO) has been reported as the rate-limiting step during S^0 recovery (Xu et al., 2012). Denitrification may have occurred in the HLFCRs as an average reduction of 24% and 71% in nitrate (NO_3^-), 73% and 89% in nitrite (NO_2^-) was observed in single (4-days HRT) and double stage operations, respectively. This suggested the possibility of their use as electron donors by chemolithotrophic (heterotrophic) SOB (Moraes et al., 2012). However, the majority of studies using other biological reactor configurations such as expanded granular sludge bed (EGSB), upflow anaerobic sludge blanket (UASB) and upflow anaerobic filter (UAF) achieved near-complete SO, particularly those that dosed the reactors with O_2.

Elemental analysis of the harvested yellow-cream FSB for carbon (C), nitrogen (N), hydrogen (H) and sulfur (S) content showed that the dried FSB from single-stage treatment at 4-days HRT contained 18.7–28.2% C (21.8% average), 0.59–1.47% N (1.0% average), 1.65–3.79% H (2.8% average), and 0.44–2.00% S (1.2% average). The harvested FSB had a yellow-cream colour characteristic of S^0. The results were comparable to those reported by Horn et al. (2022) who recovered 8–11% S and 38–58% C from one HLFCR, and 62–77% S and 6.1–6.2% C from a second HLFCR, both operated at 4-days HRT. The low S^0 content may have been due to the harvesting of the formed organic C matrix while only partially formed, and/or the low relative abundance (RA) of SOB, and/or the (re)reduction of the S^0 from the biofilm. Additionally, the subsequent high sulfide concentrations in the bulk liquid may have inhibited SRB as they were within the inhibitory range (64–448 mg S^{2-}/L) reported in literature at different pH levels (Okabe et al., 1995). However, no BSR inhibition was observed at total sulfide concentrations up to 280 mg/L at pH 7 ± 0.2 while treating synthetic wastewater with high sulfate concentration (Huang and Khanal, 2004; Khanal et al., 2003).

The increased oxygen mass transfer into the bulk liquid after the disruption of FSB did not negatively impact sulfide reduction as the oxygen was consumed by oxidation of the sulfide present into colloidal S^0 and possibly $S_2O_3^{2-}$ and polysulfide (S_n^{2-}). This made it possible to maintain anoxic conditions (−366 <ORP<−322 mV) in the bulk volume of the HLFCRs until the biofilm reformed. This was slightly higher than −410 to −350 mV optimal range for SO reported by Marais et al. (2020) and lower than −275 to −265 mV reported by Huang and Khanal (2004) and Khanal et al. (2003) treating synthetic wastewater. However, if there is insufficient sulfide in the bulk liquid or the biofilm takes too long to reform dissolved oxygen could increase, promiting complete oxidation of formed sulfur compounds back into SO_4^{2-} and ultimately an increase in redox potential and reactor failure (Marais et al., 2020).

3.1.1.1. Increased hydraulic retention time. Single-stage treatment at 8-days HRT in this study achieved the removal of 1042–1191 mg SO_4^{2-}/L (54–61% removal) and 910–913 mg S^{2-}/L (90–94% removal). In comparison to operating at 4-days HRT, these results demonstrated an insignificant ($p > 0.05$, F test) decrease of 5–16% in BSR, and a significant ($p < 0.05$, F test) increase of 33–47% in SO, 18–36% in NO_3^- and 7–15% in NO_2^- reduction. It is also plausible that NH_4^+ accumulation may have been partly due to dissimilatory reduction of NO_3^-/ NO_2^- from protein hydrolysis to NH_4^+ by chemoorganotrophic bacteria (Mpofu et al., 2022). The results were in close agreement with the 183 and 184 mg S^{2-}/L.day removal reported by Mooruth (2013) and Xu et al. (2012) using a linear flow channel reactor (LFCR) and an expanded granular sludge bed (EGSB) reactor for the treatment of synthetic wastewater and acid mine water, respectively. However, these studies used lactate as a source of readily available carbon unlike this study that only used BHE. In terms of BSR, previous studies achieved higher BSR of 980–5975 mg SO_4^{2-}/L (64–100% removal) due to a longer HRT and higher SO_4^{2-} feed rates (Table 1).

The composition of harvested the yellow-cream FSB was 10.8–14% C (12.4% average), 1.5–1.9% N (1.7% average), 1.9–4.4% H (3.1% average), and 26–39.6% S (32.8% average). A sulfur mass balance over the system at optimum operating conditions (8 days HRT, pH = 7.0–7.8, ambient temperature and −366<ORP<−322) indicated that 16–25% of the sulfur in the feed was recovered as S^0 in the biofilm and 36–56% was unaccounted for. Horn et al. (2022) reported a sulfur recovery of 6.9–10.2%, with 39–42% unaccounted for. On the other hand, Mooruth (2013), Marais et al. (2020) and Xu et al. (2012) reported 92%, 30% and 72% sulfur recovery respectively, treating low-salinity synthetic wastewater. The majority of the "missing" sulfur can be accounted for by colloidal S in the bulk volume, as evidenced by the milky appearance, with minor fractions as other short-lived sulfur species (thiosulfate and polysulfides). Loss of gaseous H_2S to the atmosphere was assumed to be negligible due to the absence of the distinctive odour and this was consistent with the detailed analysis by Mooruth (2013). Sulfur oxidation was predominantly responsible for sulfide removal as the final bulk liquid pH was alkaline (pH = 7.3–7.5). The lack of turbulent mixing in the reactor contributed to the insignificant evolution of H_2S. This was advantageous as H_2S is foul-smelling and hazardous.

3.1.2. Two stage treatment

Shifting to two-stage pre-treatment using two HLFCRs in series both operating at 4-days HRT achieved effluent concentrations of 902–1001 mg SO_4^{2-}/L (944–1043 mg SO_4^{2-}/L; 49–54% SO_4^{2-} removal) and 43–111 mg S^{2-}/L (861–929 mg S^{2-}/L; 89–96% S^{2-} removal). In comparison to single-stage treatment at 4-days HRT, this translated to an insignificant ($p > 0.05$, F test) and significant ($p < 0.05$, F test) additional removal of 54 mg SO_4^{2-}/L (5.3% removal) and 364 mg S^{2-}/L (82% removal) on average, respectively. However, compared to single-stage treatment at 8-days HRT, there were insignificant differences ($p > 0.05$, F test) of 2–6% in the S^{2-} removal and 0–21% in BSR. Therefore, second-stage treatment mainly promoted additional SO and minimal BSR while operating at 4-days HRT. This was in agreement with observations made by Horn et al. (2022) who observed improved SO (84% removal) in a second HLFCR in series and a lack of BSR after 56 days of operation. Nonetheless, the authors later observed an improved BSR and achieved an overall average BSR of 65–70% and almost complete S^{2-} removal. The results were comparable to those achieved in this study and in a study by Genschow et al. (1996) who reported 600–680 mg SO_4^{2-}/L BSR (55–58% removal) using two stage anaerobic treatment of TWW.

Similar to single-stage treatment at 4-days HRT, the composition of FSB had low S content of 1.0–1.4% (1.2% average), 1.3–1.8% N (1.5% average) and 1.3–1.8% H (1.5% average) and a higher C content of 14.4–15.7% (15% average).

3.1.3. Performance of hybrid linear flow channel reactors in the degradation of b/ovine tannery effluent

The desirable outcome from the application of HLFCR was mainly the reduction of sulfur species (HS^- and SO_4^{2-}) and metals that are toxic to AD while maintaining sufficient residual nutrients. The HLFCRs achieved reduction efficiencies of 47–71% total COD (COD_t), 34–56% total VOA (acetic acid equivalent), 10–40% total organic carbon (TOC), and 7.0–61% NH_4 after single-stage pre-treatment. This meant 29–53% COD_t and 60–90% TOC were still available for AD. As expected, higher reduction efficiencies of 62–81% COD_t, and 19–55% TOC were obtained after two stage pre-treatment as it allowed for an increase in HRT. These were higher than the 15–44% reduction in soluble COD (COD_s) reported by Horn et al. (2022) after two stage treatment and comparable to the 59–69% COD reduction achieved by Genschow et al. (1996) using a BSR system during treatment of TWW. However, an increase of 40–234% in

Table 1
Studies on the biological sulfate removal from wastewater.

Reactors	1 stage UASB/ STR/TR	1 stage LFCR	2-stage EGSB + aeration tank	1 stage HLFCR	3-stage 2xUFHR+	1-stage STR	1-stage UAF	HLFCR
Scale	Pilot	Lab-scale	Lab-scale	Lab-scale	Lab-scale	Lab-scale	Lab-scale	Pilot-scale
Reactor volume (ℓ)	2000/2000/1500	2.13	4&5	2.0	1.8/1.2 & 0.8	1	4.5	8.1
Pre-treatment	Biological	Biological	Biological	Biological	Biological	Biological	Biological	Biological
Substrate	TWW	Synthetic	Synthetic	BHE	pTWW	Synthetic	Synthetic	BHE
Operation mode	Continuous	Continuous	Continuous	Continuous	Continuous	Batch	Continuous	Continuous
HRT (days)	4	4	0.75	4	0.44	0.5–5	3/7	4 & 8
Temperature (°C)	Ambient	28	30	Ambient	30	35	35	Ambient
pH	7.4–8.2	–	–	7.0–7.7	6.0–9.0	8.0	–	7–
ORP (mV)	–	−350 to −410	–	−475 to −380	−320 to −220	–	−300 to −250	−366 to −322
Influent COD_t (g/ℓ)	5.32	0.70	3.00	17–28	2.5–3.9	–	10–18	22.8 ± 3.7
COD_t removal (%)	75–95	–	10–88	–	91–92	–	–	47–81
Influent COD_s (g/ℓ)	–	–	–	7–10	–	–	–	–
COD_s removal (%)	–	–	–	15–44	–	–	–	–
Influent SO_4^{2-} (g/L)	1.80	1.00	1.00	1.95	2.85	1.0–10	1/3/6	1.95 ± 0.31
BSR range (mg/L)	250–600	669–820	170–373	513–1049		–	975–5975	745–1093
BSR (%)	60–80	64–97	49–99	60–80	64–100	22–87	>98	38–78
Sulfide removal (%)	–	95–100	82–99	>97		–	–	50–94
S^0 recovery (%)	–	30	15–72	6.9–10	63–66	–	–	0.44-40
References	Boshoff et al. (2004)	Marais et al. (2020)	Xu et al. (2012)	(Horn et al., 2022)	Sabumon (2008b)	Oyekola et al. (2010)	Huang and Khanal (2004)	This study

BSR = biological sulfate removal; COD_t = total chemical oxygen demand; Cod_s = soluble COD.; EGSB = expanded granular sludge bed reactor; HLFCR = hybrid linear flow channel reactor; Lab = laboratory; LFRC = linear flow channel reactor; ORP = oxidation reduction potential; STR = stirred tank reactor; TWW = tannery wastewater; pTWW = primary treated; TWWTR = trench reactor; UAF = upflow anaerobic filter; UFHR = up flow hybrid reactors.

total volatile organic acids (VOA_t) (1905–4549 mg/L) and 75–231% in NH_4 (156–294 mg/L), which was theoretically attributed to increased hydrolytic activity. This was undesirable as high NH_3 (53–1450 mg NH_3/L) and VOA_t (4000–6900 mg/L) concentrations inhibit AD (Mpofu et al., 2021). Nonetheless, the differences in COD and TOC reduction efficiencies between single-stage and two-stage pre-treatment were insignificant (F test, $p > 0.05$).

Coagulation pre-treatment studies by Aboulhassan et al. (2008), Song and Williams (2004) and Song et al. (2001) reported equivalent reduction efficiencies in the range of 30–71% COD, 38–69% suspended solids (SS), 74–99% Cr, 79–100% total sulfide and/or 85–86% colour during coagulation studies. This study also demonstrated the feasibility of reducing the concentration of K, Na, Ca, Mg, Mn, Pb, Fe, and Cr through precipitation as metal sulfides and/or attached to suspended solids (SS) that settled to the bottom of the reactor. However, due to the complexity of biochemical reactions taking place, it was not possible to derive a definitive relationship between SO_4^{2-} reduction and COD, VOA_t, TOC, sulfide and/or metals reduction.

The concentration of typical toxicants: VOA, metals (K, Na, Fe, Ca, Mg, Cu, and Cr) and the COD: SO_4^{2-} ratio (>5) in the HLFCR effluent were mostly theoretically below the inhibitory range for AD (Mpofu et al., 2021). However, the respective VOA:Alk ratios of HLFCR effluents were theoretically above optimum (>0.3–0.4) while the C:N ratios were below optimal (6–9) (Berhe and Leta, 2018). The maintenance of pH at 7 ± 0.5 in the AnSBR was likely to abate inhibition by promoting the presence of NH_4^+ and a fair distribution of H_2S and HS^-. Nonetheless, single-stage (8-days HRT) and two stage HLFCR effluent characteristics were likely to lead to higher CH_4 yields during AD in comparison to AD of the raw BHE. The use of HLFCR as a pre-treatment step was effective in removing S^{2-} and SO_4^{2-} as S^0 and other potential toxicants rendering the effluent amenable for AD.

3.2. Performance of the anaerobic sequential reactor

3.2.1. Biomethane recovery and anaerobic biodegradability of b/ovine tannery effluent

The cumulative biogas (75–450 mL/COD_{added}) and CH_4 yields (41–243 mL/COD_{added}, and 64–461 mL/$COD_{consumed}$) (Table 2) obtained in this study were comparable to 75–420 mL biogas/$gCOD_{added}$, 0.21–270 mL CH_4/$gCOD_{added}$ and 300–360 mL CH_4/$COD_{consumed}$ to those reported in previous studies treating pre-treated TWW (Mpofu et al., 2021). Operating at 50 rpm under continuous mixing achieved superior results for both single and two-stage pre-treated BHE compared to intermittent mixing and continuous mixing at 100 rpm (Table 2). The digestion of pre-treated BHE from single-stage (8-days HRT) and two-stage configurations resulted in higher CH_4 yields of 238 and 243 mL CH_4/$gCOD_{added}$, respectively. Horn et al. (2022) achieved 38.4 mL CH_4/$gCOD_{consumed}$ from pre-treated BHE (5300 mg COD_s/L) using two a two-stage HLFCR configuration. In contrast to this study, the authors did not use an inoculum acclimated to pre-treated BHE. The AD of raw BHE with a higher influent COD and TOC yielded 1.6 mL CH_4/$gCOD_{added}$ in unmixed reactors while operating at 100 and 200 rpm under continuous mixing yielded 21.3 and 4.7 mL CH_4/$gCOD_{added}$, respectively.

In terms of B_0, a further 31–63% COD_t, 21–60% TOC, 74–81% SO_4^{2-}, and 39–82% S^{2-} reduction were achieved. However, there was a 75–304% increase in S^{2-} concentrations while operating at 50 rpm and 12 h mixing time. These conditions also led to a 49–56% decrease in VOA concentration while other operating conditions led to a 30–310% increase in VOA. Mpofu et al. (2022) reported the syntrophic degradation of VOAs by SRB and the inhibition of methanogens that led to the accumulation VOAs. The reduction in SO_4^{2-} in all reactors demonstrated the coexistence of SRB and methanogens, while the simultaneous NO_2^-, NO_3^- and S^{2-} decrease supported the hypothesis that they were readily utilised as electron donors by SOB. This was also evidenced by the high

Table 2
Process efficiency of anaerobic sequential batch reactors treating raw/pre-treated effluent and settled solids.

Substrate type and mixing conditions (rpm and hrs)	Specific methane generation ($mL/gCOD_{consumed}$)	($mL/gCOD_{added}$)	Biogas quality (average % CH_4)	Biodegradability (%COD reduction)
RW–BHE 0 rpm	2.16	1.55	14.9	(85)
RW–BHE 100 rpm, 24 h	25.2	21.3	46.2	(72)
RW–BHE 200 rpm, 24 h	7.18	4.65	26.8	(65)
PT–BHE 0 rpm	111	85	29.4	76
PT–BHE 50 rpm 24 h	225	128	62.0	57
PT–BHE 100 rpm 24 h	64	41	55.9	61
PT–BHE 50 rpm 12 h	104	47	34.3	45
****PT–BHE 50 rpm 24 h**	314	238	52.3	54
2 PT–BHE 50 rpm 24 h	321	243	58.9	53
2 PT–BHE 50 rpm 12 h	159	70	38.5	44
Settled solids 0 rpm	ND	*88	38.4	ND

*mL/gVS_{added}; () = soluble COD; ND = not determined; RW–BOTE = raw bovine-ovine tannery effluent; PT–BOTE = single-stage pre-treated effluent (4-days HRT); 2 PT–BOTE = two stage pre-treated effluent; **PT–BOTE = single-stage pre-treated effluent (8-days HRT); rpm = revolutions per minute hrs = hours.

H_2S content (>10000 ppm) in the biogas. Mpofu et al. (2022) also made a similar observation, together with the formation of a conspicuous white-yellowish layer of S^0 at the interface of the head-space and bulk liquid during BMP experiments. Therefore, mixing conditions and BHE pre-treatment had an impact on CH_4 yields and B_0. Schenk et al. (1999) and Wiemann et al. (1998) treating BHE also demonstrated the feasibility of removing 70–86% H_2S using stripping and achieved 9–15% improvement in B_0 and/or 40–90% increase in biogas production. Similarly, other pre-treatment studies on TWW using coagulation by Achouri et al. (2017), Song et al. (2003) and Vijayaraghavan and Murthy (1997) observed improved B_o, increased biogas production, reduction in HRT, and/or increased bioreactor capacity.

The majority of the metals in the AnSBR effluent were also below the inhibitory concentration ranges reported in literature except for Na (1184–7696 mg/L) (Mpofu et al., 2021). The AnSBRs may have experienced a deficiency of critical metabolic enzyme co-factors Ni, Mg, Zn, Co, Cu and Fe (Table 3). The AnSBRs may have operated under inhibited steady-state conditions, particularly due to inhibitory S^{2-} (68–308 mg/L) and NH_3 (176–461 mg/L) levels in the AnSBR effluent. Generally, inhibitory concentrations vary due to the different operating conditions, the complexity of biochemical reactions, microbial community composition, and degree of microbial acclimation. Mesophilic bioreactors have highly diverse and complex methanogenic communities, making the systems more adaptable, and resilient than psychrophilic and thermophilic AD, This allows mesophilic reactor to function at inhibited steady state conditions (Deng et al., 2014).

3.2.1.1. Impact of mechanical mixing. Continuous mixing (24hrs/day) at 50 rpm using the four blade marine impeller significantly improved (F test, $p > 0.05$) the cumulative CH_4 yield by 33–65% and 63–71% compared to unmixed and intermittently mixed AnSBRs, respectively. This demonstrated the positive impact of continuous mechanical mixing conditions on the rheology and the different biochemical reactions taking place in the AnSBRs. Similarly, a study by Kibangou et al. (2021) treating ostrich TWW also reported higher CH_4 yields during continuous mixing over intermittent mixing. The authors observed the preferential selection of *Methanosarcina mazei* which made up 3.6–11% and *Methanosarcina soligelidi which made up* 10.9–21% of the methanogen population. *Methanosarcina* archaea are widely reported as being metabolically versatile, highly resistant to NH_3 and VOAs inhibition and can facilitate hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis (Amin et al., 2021). The small coccoid morphology of methanogens such as *Methanosarcina* renders them less susceptible to impeller shear forces than bacilli and larger cocci.

However, CH_4 yields from unmixed AnSBRs were higher than those achieved by intermittent mixing at 50 rpm (12 h) and continuous mixing at 100 rpm (Fig. 3A and B), suggesting that mixing may have (i) played a role in promoting the transfer of toxicants (S^{2-}, NH_3 and VOAs), and/or (ii) that there was disruption of synergies and destruction of functional biomass due to excessive continuous mixing. It was postulated that the presence of micro-niches in unmixed AnSBRs may have protected microbes against high levels of inhibitory substances in the bulk liquid. This suggested that sedimentation in the unmixed AnSBR may have also provided closer microbial consortia proximity (juxtapositioning), essential in syntrophic microorganisms such as syntrophic SRB and hydrogenotrophic methanogens (Kim et al., 2002). Long-term sedimentation in unmixed reactors led to higher B_0, 85 and 76% COD reduction for raw and pre-treated effluent (Table 2). However, the disruption of these micro-niches and incomplete mixing of AnSBRs due to intermittent mixing may have led to lower B_0 and CH_4 yields. The AD of pre-treated BHE from the 2 stage configuration achieved insignificantly higher (2%) CH_4 yields compared to effluent from single-stage 8-days HRT. In contrast, two-stage and single-stage (8-days HRT) configurations achieved significantly higher CH_4 yields compared to single-stage HLFCRs operating at 4-days HRT. As expected, all pre-treated BHE had ideal characteristics for AD and achieved higher CH_4 yields. This proved the positive impact of pre-treatment.

3.2.2. Biomethane recovery from hybrid linear flow channel reactor settled solids

As expected, settled solids (SS) from the HLFCRs were unsuitable for AD due to high concentrations of metals (Fig. 3C) and most likely limited TOC that was not readily hydrolysable (Mpofu et al., 2021). Nonetheless, their AD in unmixed AnSBRs suffered a lag phase of about 10 days (Fig. 3C) and may have operated under steady state conditions thereafter leading to longer retention time (RT). The 88 mL CH_4/gVS (38.4% average CH_4) yield achieved in this study after 110 days was comparable to the 89 mL CH_4/gVS (40–57% CH_4) and 84 mL CH_4/gVS (56% average CH_4) reported by Akyol et al. (2014) and Mpofu et al. (2020b) after 40 and 108 days while digesting tannery wastewater sludge (TWS) at ISR = 0.1 and 4, respectively. Another study by Mpofu et al. (2020) investigating the effect of initial pH (6–9) and operating temperature (23–40 °C) during the AD of TWS reported CH_4 yields in the range 12.5–176 mL CH_4/gVS (40–58% average CH_4), 25.4–78.7% VS, 3.6–66.4 %TS and 22.7–51.7% COD reduction after 108 days. Efforts to improve CH_4 yields by Agustini et al. (2018a) and Agustini et al. (2018b) using codigestion of TWS with leather shavings resulted in lower CH_4 yields of 15 (57% average CH_4), and 8.2 mL CH_4/gVS after 200 and 170 days RT, respectively.

3.3. Performance of the integrated biological system

The integrated system's optimum operating configuration was single-stage (8-days HRT) HLFCR and 50 rpm continuous mixing or two stage HLFCR (4-days HRT in each reactor) and 50 rpm continuous mixing. These operating conditions achieved the removal of 80–91%

Table 3
Concentration of bovine/ovine tannery effluent and sludge at different stages of the integrated treatment system and South African discharge standards.

	Beamhouse	HLFCR (mg/L)		HLFCR SS	Digested SS	AnSBR (mg/L)		Irrigation Standard (mg/L)		Land application (mg/kg)	
	Effluent (mg/L)	Single-stage	two- stage	(mg/kg)		*50 rpm 24 h	50 rpm 24hrs	South Africa	Other countries	South Africa	Other countries
TS	32500 ± 1650			123000 ± 424							
COD	22780 ± 3737	8723 ± 1480	6373 ± 1681			3790 ± 154	2505 ± 276	75–5000	40–5000		
TOC	4603 ± 10.6	3748 ± 707	2925 ± 811			2520 ± 14.1	1510 ± 78				
Alk	3780 ± 424	3892 ± 583	1405 ± 302			2003 ± 539	2075 ± 332				
VOA	1362 ± 31.4	721 ± 104	2971 ± 1171			2465 ± 21	2284 ± 98				
Sulfate	1945 ± 313	907 ± 240	956 ± 38			179 ± 34	205 ± 49		0–1000		
Sulfide	1119 ± 17.7	461 ± 59.6	81 ± 29			77 ± 10.4	68 ± 3.5				
NH_4	43.6 ± 12.4	51.9 ± 16.5	232 ± 53.8			444 ± 55.6	232 ± 19	0–3	0–50		
pH	16.2	13.1	7.44			7 ± 0.5	7 ± 0.5	5.5–9.5	5–10		
PO4	16.2 ± 1.0	7.90 ± 2.38	5.25 ± 4.88			4.35 ± 0.07	8.15 ± 5.7	10	0.2–30		
NO_3^-	11.5 ± 0.9	10.5 ± 3.14	4.04 ± 1.57			3.9 ± 0.14	5.7 ± 0.1	15	0–50		
NO_3^-	4.5 ± 1.2	1.26 ± 0.23	0.5 ± 0.29			0.70 ± 0.01	0.65 ± 0.01				
TN	1163 ± 212	811 ± 138	680 ± 165				610 ± 9.2		15–70		
Cl	9723 ± 48	8268 ± 2907	8950 ± 69			5854 ± 47	7850 ± 495	0.25 as Cl_2	0–1000		
Na	2141	2014 ± 830	4827 ± 1231	21.3 ± 11	40.2 ± 5.76	2769	2378		50–230		
As		<0.01	<0.01	1.06 ± 0.01	1.05 ± 0.32	<0.01	<0.01		0.05–0.4		10–75
Se		<0.01	0.02 ± 0.01	0.26 ± 0.07	0.39 ± 0.06	0.05	<0.01		0.02–0.05		36–100
Mo		<0.01	<0.01	0.28 ± 0.01	1.48 ± 0.33	<0.01	<0.01		0.01–0.05		18–40
Si	0.20	0.14 ± 0.02	1.7 ± 0.88	1.3 ± 0.14	1.12 ± 0.07	5.67	1.98				
K	31	19.8 ± 6.93	201 ± 69	0.4 ± 0.17	1.69 ± 0.1	56.4	34.3				
Ca	660	485 ± 206	425 ± 229	250 ± 18	61 ± 14.5	141	125				80–400
Mg	1.34	0.06 ± 0.01	6.23 ± 2.14	6.3 ± 0.75	2.91 ± 0.59	13.4	6.31		50–120		
Ni		0.022 ± 0.005	0.057 ± 0.05	3.38 ± 0.39	5.87 ± 2.34	0.14	0.095		0.1–4.0	420	40–420
Al	0.06	0.13 ± 0.07	0.63 ± 0.27	8245 ± 2347	3451 ± 739	0.05	0.13				
Cu		<0.01	0.15 ± 0.09	14.6 ± 0.91	28.4 ± 3.5	0.17	0.06		0.2–6.0	1500–4300	300–4300
Fe	0.03	<0.01	0.08 ± 0.03	740 ± 2.66	2113 ± 343	0.04	0.07		0.2–5.0	0.5–3.0	
Zn	0.13	0.11 ± 0.07	0.11 ± 0.03	29.5 ± 2.58	122 ± 31	0.18	0.25		2.0–20	2800–7500	1000–7500
Sr	1.57	1.1 ± 0.54	2.3 ± 0.95	669 ± 34.7	196 ± 40.5	0.59	0.70				
Ba	0.22	0.16 ± 0.08	0.19 ± 0.08	78.8 ± 2.29	41 ± 9.35	0.06	0.09				
Cd	<0.01	<0.01	<0.01	0.02 ± 0.005	0.09 ± 0.02	<0.01	<0.01		0.01–0.1	40–85	3–85
Pb	0.018	0.11 ± 0.03	0.11 ± 0.02	1.78 ± 0.09	5.29 ± 1.62	<0.01	<0.01			300–840	150–1000
B	0.54 ± 0.07	0.58 ± 0.07	0.54 ± 0.04	24.3 ± 3.46	16 ± 3.0	0.56	0.57				
Cr	<0.01	<0.01	<0.01	9.1 ± 0.76	2030 ± 446	0.03	<0.01		0.05–2.0	1200–3000	50–3000
Hg	<0.01	<0.01	<0.01	0.03 ± 0.001	0.32 ± 0.17	<0.01	<0.01	0.001–0.01		15–55	0.15–57

**PT–BOTE = single-stage pre-treated effluent (8-days HRT).
AnSBR = anaerobic sequential batch reactor.
HLFCR = hybrid linear flow channel reactor.
SS = settled sludge.
rpm = revolutions per minute.

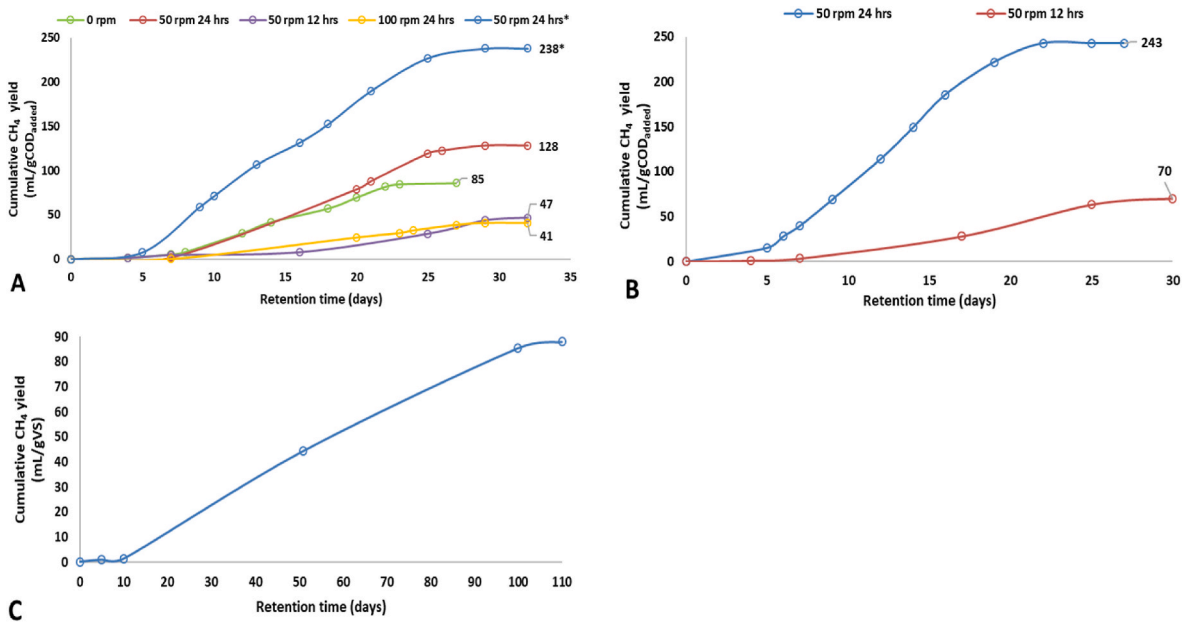

Fig. 3. Cumulative methane yields from (A): single-stage – 4 days and *8-days HRT, (B): two stage pre-treated bovine-ovine tannery effluent and (C): settled solids from hybrid linear flow channel reactors at different mixing conditions.

total COD, 78–98% TOC, 89–91% SO_4^{2-}, 92–93% S^{2-}, 50–73 PO_4^{2-}, 48–60% total nitrogen, 60–72% NO_3^- and 84–86% NO_2^- (Table 3). This was comparable to the 40–99% SO_4^{2-}, 82–99% S^{2-} reduction (Section 3.1) and 43–95% (68–88% soluble COD) reported in previous studies (Mpofu et al., 2021). However, the hydrolysis process particularly in the second HLFCR (two-stage configuration) and/or in AnSBRs led to a 68–81% and 162–400% increase in VOAs and ammonia, respectively. A number of metals seemingly increased in concentration partly due to their presence in the inoculum used and the different physical, chemical and/or biochemical reactions taking place. Nonetheless, an overall decrease of 79–81% in Ca, 56–62% Sr, and 57–72% Ba was recorded.

The system achieved the production of 241 ± 4 mL $CH_4/gCOD_{added}$ from BHE and 88 ± 2 mL CH_4/gVS_{added} from treating SS from HLFCR. The final treated effluent met many of the irrigation standards for bovine leather-producing countries (Brazil, China, Egypt, Ethiopia, India, Mexico, South Africa, South Korea and Turkey) except for Na, Cl, NH_3/NH_4, total nitrogen and COD. Similarly, the digestate met the stipulated standards for its application on land in most countries except for Cr. Chrome (III) is insoluble in water and hence it attaches and settles with solids. In the South African context, the treated BHE met the standards for irrigating up to 50 m^3/day and the A1b sludge guidelines (Department of Water Affairs, 2013; Snyman and Herselman, 2006). Mpofu et al. (2022) recommended further treatment and/or dilution of TE for reuse e.g. in soaking, de/liming and/or tanning. Further treatment processes would promote the recovery of VOA (2284–2465 mg/L) and NH_4 (232–444 mg/L) that were still present in high concentrations (Table 3).

3.4. Kinetic study of anaerobic sequencing batch reactor performance

The kinetics of AnSBRs were evaluated by fitting the modified Gompertz, first-order, Logistic, and Cone, models onto the cumulative CH_4 yield data using non-linear regression methods. The determination of kinetics will aid in the full scale process and reactor design and in process simulation. The Cone (Adj $R^2 > 0.988$), Logistic (Adj $R^2 > 0.986$) and first order (Adj $R^2 > 0.902$) kinetic models displayed the best fit of the experimental cumulative CH_4 yields. However, the first order, Chen & Hashimoto ($0.825 < \text{Adj } R^2 < 0.984$) and modified Gompertz ($0.343 < \text{Adj } R^2 < 0.993$) models yielded impractical values of ultimate cumulative CH_4 yields (A) and/or maximum CH_4 production rate (microbial specific growth rates) (μ_m) (Table 4). The first order and C&H models displayed similar straight line trends (Fig. 4) leading to high A and very low μ_m. The modified Gompertz model significantly underestimated A due to its exponential rise to a maximum regression. The Cone and Logistic model were good at regressing sigmoidal cumulative CH_4 yields that included the lag, exponential rise and plateau phases generally displayed by inhibited steady state bioreactors treating tannery effluents or sludge (Mpofu et al., 2020b, 2020, 2021, 2022).

The kinetic constants obtained in this study were μ_m = 2.88–19.5 mL $CH_4/gCODd$, reaction rate (K) = 0.04–0.08 day^{-1}, and lag phase (λ) = 8.1–14.9 days depending on the pre-treatment configuration and mixing conditions. The highest μ_m (12.7–19.5 mL $CH_4/gCODd^{-1}$) and K (0.06–0.08) were obtained in continuously mixed AnSBRs operating at 50 rpm (Table 4). The 2 stage pre-treated BHE achieved higher values probably due to relatively lower levels of inhibitors and higher levels VOAs. However, continuously mixing at 100 rpm led to the lowest reaction kinetics (A, μ_m and K) due to the negative impact of excessive mixing (section 3.2.1.1). The kinetic constants obtained in the unmixed AnSBR were relatively similar (F test, $p > 0.05$) to those obtained in intermittently mixed reactors (Table 4). Similarly, kinetics obtained in mixed AnSBRs were comparable to μ_m = 0.17–17.5 mL $CH_4/gVSd$, K = 0.025–0.27 day^1 and λ = 3–27 days reported by Mpofu et al. (2021b) while digesting ostrich tannery effluent. Additionally, BMP tests on raw BHE by Mpofu et al. (2022) reported higher μ_m (= 24.6–74.2 mL $CH_4/gVSd^{-1}$) but equivalent K (= 0.08 day^{-1}) and λ (= 5–12 days). This demonstrated the positive effect of micro niches resulting from sedimentation in unmixed bioreactors (section 3.2.1.1). The K:μ_m ratios (>1) in this study demonstrated that methanogenesis was the rate limiting step due to its inhibition.

The kinetics of the AD of settled solids were μ_m = 3.76 mL $CH_4/gCODd^{-1}$, K = 0.02 and λ = 39 days. This was similar to μ_m = 2.04–5.48 mL $CH_4/gVSd^{-1}$ obtained by Sri Bala Kameswari et al. (2014) and K = 0.0185–0.0239 d^{-1} reported by Thangamani et al. (2010, 2009) during the co-digestion of tannery sludge with tannery solid wastes. In contrast, Mpofu et al. (2020, 2020b) reported lower K = 0.008–0.012 day^{-1} and μ_m = 1.32–2.84 mL $CH_4/gVSd^{-1}$ during the AD of TWAS that was recalcitrant nature. The authors further reported improved K = 0.011–0.0291 d^{-1} and μ_m = 4.37–18.1 mL $CH_4/gVSd^{-1}$ after codigestion with slaughterhouse sludge.

Table 4
Kinetic parameters and goodness of fit obtained from evaluated models.

Runs	Model	Kinetic parameters					$AdjR^2$	p-value Prob > F	AIC	RMSE
		A	μ_m	ʎ	K	n				
		($mLCH_4/gCOD$)	($mLCH_4/gCODd^{-1}$)	(d)	(d^{-1})					
0 rpm (1 stage)	Cone	104	ND	ND	0.06	3.45	0.994	0.497	112	1.65
	Logistic	90.2	6.42	8.1	ND	ND	0.991	0.473	120	1.94
	First order	95	ND	ND	0.05	ND	0.974	0.488	208	9.79
	C & H	9.5E5	0.31	ND	74674	ND	0.953	0.455	167	17.0
	Gompertz	85	0.93	32.1	ND	ND	0.924	0.022	241	17.9
50 rpm 12 h (1 stage)	Logistic	53.8	3.02	14.9	ND	ND	0.992	0.482	93.2	0.85
	Cone	60.1	ND	ND	0.04	4.63	0.988	0.467	108	1.13
	C & H	1.2E6	0.56	ND	4.7E5	ND	0.921	0.386	170	2.64
	First order	7.9E5	ND	ND	1.7E-6	ND	0.902	0.321	178	2.94
	Gompertz	12.6	1.23	8.14	ND	ND	0.462	0.216	236	6.90
50 rpm 24 h (1 stage)	Logistic	130	12.7	13.9	ND	ND	0.999	0.498	41.6	0.41
	Cone	131	ND	ND	0.05	8.51	0.999	0.492	66.6	0.61
	C & H	9.8E4	4.0E5	ND	9.2E9	ND	0.939	0.370	215	6.14
	First order	1.8E5	ND	ND	2.4E-5	ND	0.937	0.370	215	6.13
	Gompertz	35.9	5.57	3.35	ND	ND	0.343	0.117	290	19.8
50 rpm 24 h (*1 stage)	Cone	302	ND	ND	0.06	2.37	0.994	0.491	185	3.82
	Gompertz	97.6	4.63	12.6	ND	ND	0.993	0.486	185	3.86
	Logistic	247	13.2	5.67	ND	ND	0.989	0.465	201	4.93
	C & H	1.6E4	105	ND	2.0E5	ND	0.952	0.405	242	9.37
	First order	4381	ND	ND	0.002	ND	0.962	0.401	366	9.39
100 rpm 24 h (1 stage)	Cone	46.5	ND	ND	0.05	4.59	0.996	0.237	49.8	0.47
	Logistic	42.8	2.88	12.0	ND	ND	0.996	0.482	55.1	0.51
	C & H	4.9E5	0.35	ND	1.2E5	ND	0.961	0.481	125	1.51
	First order	4.3E3	ND	ND	3.1E-4	ND	0.945	0.362	136	1.79
	Gompertz	11.2	1.83	3.52	ND	ND	0.348	0.12	215	6.16
50 rpm 12 h (2 stage)	Logistic	73.3	5.23	11.6	ND	ND	0.999	0.499	−5.33	0.19
	Cone	75.9	ND	5.18	0.05	5.47	0.998	0.483	62.5	0.60
	C & H	2.0E5	0.32	ND	1.7E6	ND	0.954	0.481	153	2.71
	First order	7.9E5	ND	ND	2.8E-6	ND	0.921	0.357	169	3.55
	Gompertz	32.0	3.60	15.0	ND	ND	0.780	0.285	263	16.9
50 rpm 24 h (2 stage)	Logistic	249	19.5	6.15	ND	ND	0.998	0.493	136	0.49
	Cone	278	ND	ND	0.08	3.11	0.995	0.492	160	4.03
	C & H	531	0.21	ND	4.77	ND	0.984	0.490	195	7.73
	First order	1.2E4	ND	ND	8.5E-4	ND	0.929	0.335	234	15.8
	Gompertz	69.5	10.5	7.05	ND	ND	0.507	0.203	286	41.6
0 rpm (Settled Solids)	Cone	101	ND	ND	0.02	2.85	0.999	0.496	−192	0.097
	Logistic	86.6	3.76	39.3	ND	ND	0.999	0.494	−19.2	0.21
	First order	120	ND	ND	0.01	ND	0.971	0.403	412	1.52
	C & H	0.12	5.6E-5	ND	0.99	ND	0.825	0.312	610	3.74
	Gompertz	45.0	9.69	20.0	ND	ND	0.846	0.167	890	13.3

μ_m = maximum CH_4 production rate constant (maximum specific growth rate of microorganisms); ʎ = lag phase; A = ultimate CH_4 yield; K = specific rate constant; n = shape factor constant; P = cumulative CH_4 yield; C&H = Chen and Hashimoto model; * = single-stage 8-days HRT.

3.5. Extension of results to full scale application of the integrated biological treatment system

The results from the integrated system were extended to a full-scale application at a medium sized tannery. It was estimated that the integrated system can potentially generate US$5559 through the recovery of an impure sulfur compound with 33% S^0 (307 kg), about 5.1 ML (ML) of biogas (67% average CH_4) and 31 tonnes of biofertiliser/composting material when treating 2.3 ML/day of BHE (Table 5). Alternatively, the biogas generated can be used to produce approximately 32.4 MWh of electricity that can be used to process 7359–17042 hides, while reducing electricity demand by 72% (Table 5). There is a potential revenue of US $25.97 and US$1062 from the sale of the recovered FSB and biofertiliser/compost for agricultural applications. Additionally, reducing the demand of coal-generated electricity and replacing the widely used conventional activated sludge process (CAS) would reduce carbon equivalent emissions by 94% (169 t CO_2 eq).

3.6. Impact of the and future perspectives

This study demonstrated that the full-scale application of biological treatment of BHE presents an opportunity for tanneries in developing countries to transition to a circular bioeconomy and net positive operations. However, wastewater treatment practitioners should be upskilled as the integrated system is a complex bioprocess that is susceptible to inhibition. Governments may assist tanneries by providing capital funding and introducing progressive policies, regulations, incentives and subsidies to support the adoption of bio-refineries. Furthermore, governments and the private sector can assist with the sustainability of bio-refineries by signing long-term agreements with tanneries for purchasing recoverables, particularly sulfur, and/or biofertiliser for agricultural application. The integration of tanneries with cattle farms and/or slaughterhouses would be vital in promoting a circular bioeconomy as both industries are interdependent. Integration will promote the use of green skins/hides and eliminate NaCl in BHE. The treated BHE can be further treated for the recovery of VOA and NH_3 that are still present in high concentrations (2284–2465 AAE mg/L and 232–444 NH_4 mg/L,

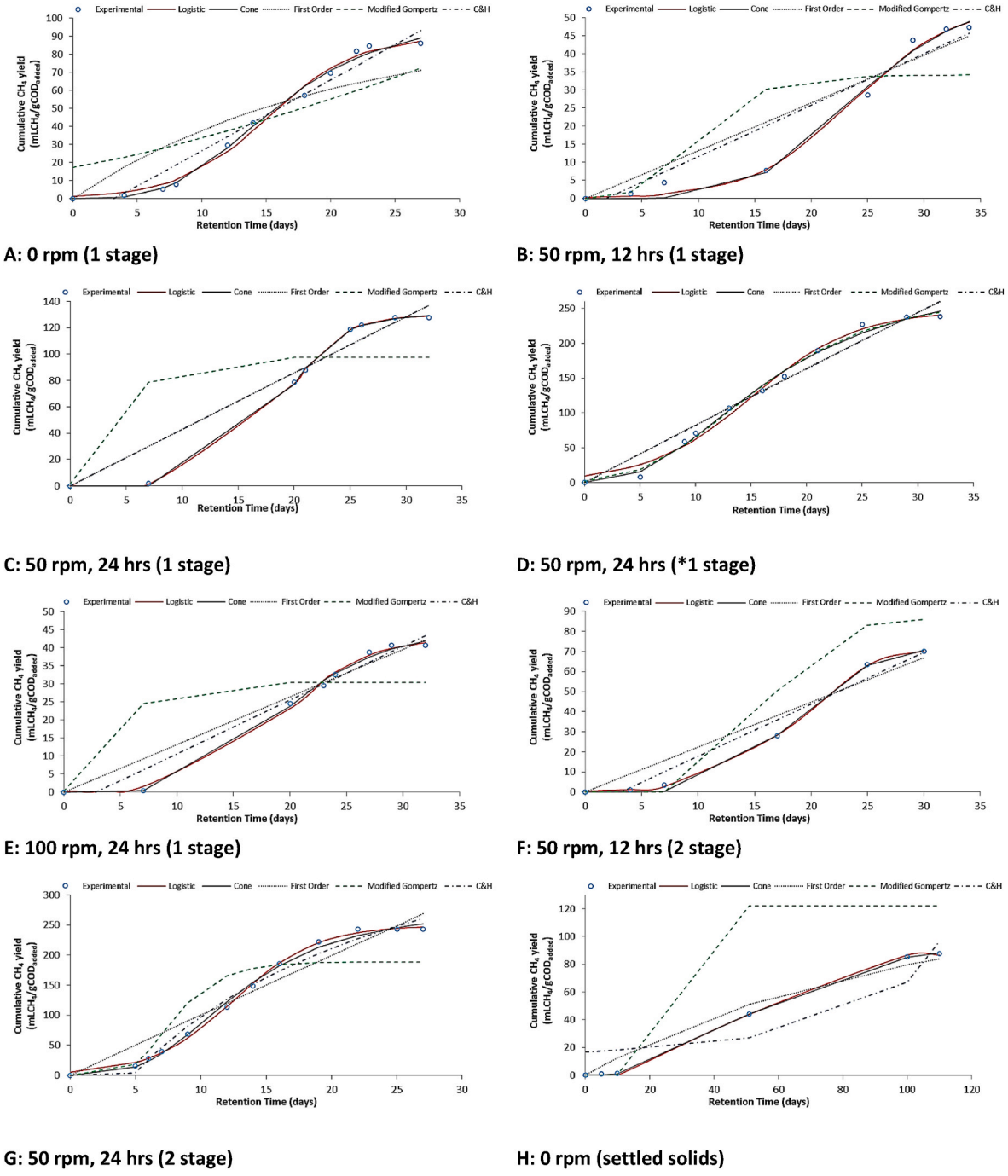

Fig. 4. Graphs depicting the experimental and kinetic model curves for cumulative methane yields during anaerobic digestion of single and 2 stage pre-treated b/ovine tannery effluent and settled solids from the hybrid linear flow channel reactor at different mixing speed and time.

respectively). This will also aid in producing better effluent quality that can be reused within the tannery and/or in other industries within an eco-industrial park and/or special economic zone.

Long term investigations to determine the operational parameters needed to promote the proliferation of optimal functional microbial consortia for bioaugmentation in an integrated treatment system are required. Furthermore, the kinetics and modelling of HLFCRs should be continued to better understand the pre-treatment process. There is also a need to modify the HLFCRs to improve the harvesting of formed FSB and/or their automation to optimise the addition of air via controlling the ORP. Additional investigations should be performed to determine if CH_4 production and/or energy consumption can be improved by employing different reactor and/or mixing configurations.

4. Conclusion

Pre-treated beamhouse effluents produced by single-stage (8-days HRT) and two-stage (4-days HRT each) HLFCR configurations exhibited the most ideal characteristics for anaerobic digestion (AD). Their treatment in continuously mixed AnSBR at 50 rpm achieved the highest yields (238 and 243 mL $CH_4/gCOD_{added}$) and kinetics (μ_m = 12.7–19.5 mL $CH_4/gCODd^{-1}$ and K = 0.06–0.08) that were determined using the Cone (Adj $R^2 > 0.988$) and Logistic (Adj $R^2 > 0.986$) models. However, the single-stage HLFCR configuration (8-days HRT) recovered the most S^0 compared to single (4-days HRT) and two stage configurations. Continuously mixed reactors at 100 and 200 rpm led to the lowest yields and kinetics due to the negative impact of mechanical shear stress. This

Table 5
Economic analysis at full scale daily application for a medium sized tannery.

Solid waste and wastewater generated	
B/ovine skins/hides processed (35% hides and 65% skins)	10250
Weight of skin/hides (tonne)	113
Weight of sludge produced (tonne)	56.5
Volume of water used (m^3)	2426
Volume of wastewater discharged (m^3)	2258
Average electricity consumption (MWh)	45.1
Carbon emissions (tonnes of CO_2 equivalent)	396
Operating costs	
Cost of municipal water (US$)	4662
Cost of wastewater disposal (US$)	3043
Cost of electricity (US$)	5261
Cost of landfill disposal of solid waste produced (excluding transport) (US$)	1047
Potential savings	
Biogas/(CH_4) generated (m^3/m^3 BHE $_{added}$)	5.1/(3.42)
Electric energy production potential (MWh)	32.4
Reduction in electricity consumption (%)	72%
Reduction in wastewater disposal (%)	[a]88%
Potential electricity cost savings (US$)	3777
Wastewater discharge potential savings (US$)	67.39
Reduction in landfill disposal of solid waste (%)	62%
Reduction in waste disposal costs (US$)	626
Reduction in carbon emissions (%)	[b]94%
Potential revenue and investment summary	
[c]Potential impure sulfur sales (US$)	25.97
Potential biofertiliser/compost sales (US$)	1062
Payback period (years)	5.3
Net present value (US$)	3 623 482
Internal rate of return (%)	18%

[a] Assuming treated effluent is reused for irrigation and other non-potable purposes.
[b] Without taking into account CO_2 released during the generation of electricity from biogas.
[c] Assuming that the process recovers 0.14 kgS^0/m^3 of treated effluent.

study demonstrated the technical and financial feasibility of a novel integrated biological system that promotes a circular bioeconomy and net positive tannery operations. Tanneries, must however promote the processing of green hides and/or separation of the soaking stream as it contributes high sodium and chlorides to the beamhouse effluent.

CRediT authorship contribution statement

A.B. Mpofu: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **W.M. Kaira:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation. **G.A. Holtman:** Conceptualization, Methodology, and, Investigation. **O.O. Oyekola:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition. **R.P. van Hille:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Funding acquisition. **P.J. Welz:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Visualization, Supervision, Project administration, and, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pamela Jean Welz reports financial support was provided by Water Research Commission.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2023.135872.

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