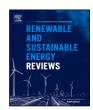
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# Review article

# Two-phase anaerobic digestion in leach bed reactors coupled to anaerobic filters: A review and the potential of biochar filters

B.A. Collins a,d,\*, C.H. Birzer a,d, P.W. Harris b, S.P. Kidd c,d, B.K. McCabe b, P.R. Medwell a,d

- a School of Mechanical Engineering, The University of Adelaide, South Australia, 5005, Australia
- <sup>b</sup> Centre for Agricultural Engineering, University of Southern Queensland, Toowoomba, Queensland, 4350, Australia
- <sup>c</sup> School of Biological Sciences, The University of Adelaide, South Australia, 5005, Australia
- <sup>d</sup> Humanitarian and Development Solutions Initiative, The University of Adelaide, South Australia, 5005, Australia

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#### ABSTRACT

Biochar addition in anaerobic digesters is an emerging technique for enhancing high-solids anaerobic digestion. Recycling of biochar can further enhance performance and reduce costs for biochar production; however, mixing biochar with feedstock and separating from digestate is impractical. A more pragmatic method of applying biochar for high-solids digestion could be coupling a leach bed reactor (LBR) with a biochar-packed anaerobic filter (AF) to form a two-phase system. Separating the anaerobic digestion process between reactors can improve process efficiency by enhancing hydrolysis and acidogenesis in the LBR and producing higher quality biogas in the AF. However, two-phase systems can be inefficient if separation of the anaerobic digestion process between reactors – referred to as phase separation – is poor. This article aims to: (i) integrate current knowledge from literature investigating batch LBR-AF systems to improve understanding of the role of different process parameters on phase separation and process efficiency; and (ii) explore the idea of biochar as a filter medium in an LBR-AF system.

Feedstocks that rapidly degrade and have ongoing VFA production are particularly suitable for phase separation in LBR-AF systems. Controllable process parameters identified as critical for phase separation and process inhibitor mitigation include co-digestion, recirculation parameters, filter media properties, inoculation method and temperature. The application of biochar in other systems highlights the potential for LBR-AF application. Future research should consider trade-offs between biogas production and digestate quality when optimising LBR-AF performance, and assess economic viability considering the additional expenses of LBR-AF systems.

# 1. Introduction

A looming energy crisis and mounting environmental concerns including climate change and waste management are motivating research and development of renewable energy technologies, such as those that harness anaerobic digestion. Anaerobic digestion is the microbial conversion of organic matter, such as agricultural or municipal wastes, in the absence of oxygen to biogas and digestate. Biogas is a combustible gas mixture containing methane that can be used as a source of renewable energy, while digestate is residual inorganic and partially digested organic matter that may be applied as fertiliser [1,2]. Various anaerobic digestion technologies have been developed to process wastes and recover these valuable by-products.

A key distinction between different anaerobic digestion technologies is the permissible total solids content (i.e., proportion of dry matter) of the substrate to be digested. Systems that process total solids contents

of <10%, 10%–15% and >15% are classified as low-solids, hemi-solid or high-solids, respectively [3]. The most common technology and thus primary focus of research has been low-solids digestion; however, high-solids digestion is an emerging technology [4] that would benefit from additional applied and fundamental research to further optimise the quality and quantity of biogas production, and digestate characteristics.

An emerging technique to enhance anaerobic digestion is the addition of biochar to anaerobic digesters. Biochar is a porous, conductive, carbonaceous material commonly used as a soil amendment. Mixing biochar with feedstock has been observed to improve the performance of batch high-solids digesters [5–7]. Recycling used biochar can further enhance anaerobic digestion [5,7,8], while reducing costs as constant biochar production is not required [5]. However, separation of biochar from digestate, and to a lesser extent mixing with feedstock, is impractical. A more pragmatic method of applying and re-using biochar

<sup>\*</sup> Correspondence to: School of Mechanical Engineering, South Australia, 5005, Australia. E-mail address: ben.collins@adelaide.edu.au (B.A. Collins).

AF	Anaerobic filter
BFS	Blast furnace slag
C/N	Carbon-to-nitrogen ratio
COD	Chemical oxygen demand
DIET	Direct interspecies electron transfer
HRT	Hydraulic retention time
IET	Indirect electron transfer
LBR	Leach bed reactor
MSW	Municipal solid waste
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
SRT	Solid retention time
VFA	Volatile fatty acids
VS	Volatile solids
UASB	Upflow anaerobic sludge blanket
WRR	Water replacement rate

for batch high-solids digestion could be coupling a biochar-packed anaerobic filter (AF) to a leach bed reactor (LBR) to form a two-phase system. An LBR is a common technology employed for batch high-solids anaerobic digestion that recirculates process liquid – known as leachate - that has percolated through organic matter (Fig. 1a). If coupled to an AF, leachate recirculates through a packed body of porous media (e.g., biochar) that supports microorganism attachment and biofilm growth (Fig. 1b). An LBR can also be coupled to other reactors for application and re-use of biochar, such as a continuous stirred-tank reactor, expanded granular sludge blanket or upflow anaerobic sludge blanket (UASB). However, the simplicity of a filter is appealing as our research context is focussed on potential application for smallscale humanitarian environments, while keeping in consideration the potential for use at larger scales. With this context in mind, exploration of the idea of coupling a biochar-packed AF to an LBR provides the motivation for review of the current knowledge on LBR-AF systems, and discussion of the potential of biochar application.

Although two-phase anaerobic digestion has been broadly reviewed considering both low- and high-solids systems [9], LBR-AF systems have been largely neglected compared with LBR-UASB systems. This is likely due to UASBs being the most widely used high-rate, low-solids reactor for treatment of domestic and industrial wastewaters [10-12]. Furthermore, compared with UASBs, AFs have been reported to have significantly longer start up times [13,14], require lower organic loading rates (OLR) for stable operation [10,15,16], and be susceptible to accumulation of non-biodegradable solids resulting in filter blockage and channelling (hydraulic short-circuiting that bypasses significant portions of the filter) [10,13]. Despite these reported drawbacks, AFs have also been observed to perform comparatively well with UASBs in terms of methane yield and content, and chemical oxygen demand (COD) reduction, when operating as single-phase reactors [16-18] or coupled to LBRs [19,20]. Comparable performance between these reactors provides further justification for a more thorough review of literature investigating LBR-AF systems.

Two-phase systems such as an LBR-AF can overcome limitations for single-phase LBRs. In a single-phase system, the entire anaerobic digestion process consisting of hydrolysis (organic matter degradation), acidogenesis (acid production), acetogenesis (acetate production) and methanogenesis (methane production), occurs in one reactor. Each of these process steps is facilitated by a unique functional group of microorganisms that have varying levels of sensitivity to environmental conditions [21,22]; e.g., methanogenesis performs best at a pH that is sub-optimal for hydrolysis [23,24]. Therefore, as the environmental

conditions of single-phase reactors are typically tailored to the more sensitive methane producing microorganisms (methanogens), performance is limited by hydrolysis [23,25]. Furthermore, as batch LBRs are typically operated with high initial organic loading, early reactor failure due to the accumulation of volatile fatty acids (VFAs) is a common issue [20,26]. Coupling an LBR with a low-solids reactor is one approach that can improve hydrolysis [25,27] and prevent failure due to VFA accumulation [20,23], while also enhancing biogas yield and methane content [28,29]. The two-phase system can enable tailoring of environmental conditions in the LBR (acidogenic reactor) to improve hydrolysis and VFA production, and conditions in the low-solids reactor (methanogenic reactor) to enhance VFA conversion to methane. Fig. 2 summarises the advantages and disadvantages of an LBR-AF system compared to a single-phase LBR. Despite the outlined benefits for twophase LBR systems, increased complexity compared to single-phase LBRs (additional reactor and associated equipment) increases capital and operating expenses. The trade-off between improved performance and increased expenses for two-phase systems can be economically unattractive [9]. Therefore, further research is needed to optimise biogas production and quality, as well as digestate quality, in order to evaluate the economic viability of LBR-AF systems.

A key factor determining the efficiency of two-phase systems is phase separation. Phase separation refers to distinct separation of the anaerobic digestion process between the reactors of a multi-phase system; i.e., for an LBR-AF system, hydrolysis and acidogenesis occurring predominantly in the LBR, and methanogenesis in the AF. Poor phase separation can limit enhancement of hydrolysis and methane production compared with single-phase LBRs; i.e., an LBR-AF system is inefficient if most methane is produced in the LBR [25,29,30]. Often, successful phase separation is not achieved in LBR-AF systems, with significant proportions of acidogenesis, acetogenesis and methanogenesis occurring in both reactors [31]. Therefore, it is crucial to understand how process parameters influence phase distribution throughout an LBR-AF system.

Although two-phase anaerobic digestion systems have been a focus in recent reviews [9,46–50], LBR-AF systems have had minimal coverage. No review has specifically integrated current knowledge from literature investigating LBR-AF systems. This review aims to fill this gap by critically reviewing literature investigating LBR-AF systems to integrate current knowledge (and identify gaps) on the role of different process parameters on phase separation and process efficiency. The potential application of a biochar filter in LBR-AF systems is also explored. To be clear, this review aims to exhaustively review literature on batch LBRs coupled to AFs; though, where appropriate, key knowledge from studies on single-phase LBRs and other relevant systems is also considered. The mini-review provided to explore the potential of biochar filters draws key knowledge from studies applying carbonaceous materials for high- and low-solids anaerobic digestion; but it is by no means exhaustive.

# 2. Process optimisation

To optimise the performance of anaerobic digestion systems there is a need to understand the influence of different process parameters. This section outlines and discusses the influence of key process parameters investigated in studies employing LBR-AF systems. The process parameters considered are temperature, AF effluent recirculation and digestate/leachate recycling, solid retention time (SRT), flow mode and hydraulic retention time (HRT), organic loading rate, AF pressure and filter media type.

# 2.1. Temperature

Although anaerobic digesters can operate at lower temperatures, mesophilic (30–40  $^{\circ}$ C) and thermophilic (50–60  $^{\circ}$ C) temperature regimes

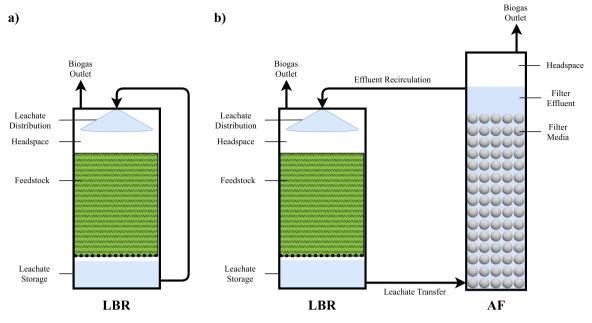


Fig. 1. Schematic of leach bed reactor anaerobic digestion systems, showing: (a) a single-phase leach bed reactor; and (b) a two-phase system consisting of a leach bed reactor coupled to an anaerobic filter.

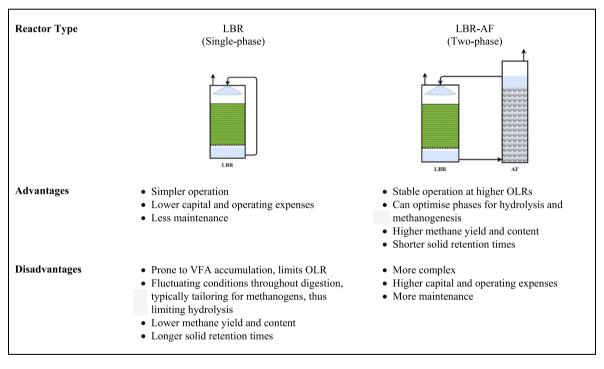


Fig. 2. Advantages and disadvantages of an LBR-AF system compared to a single-phase LBR.

are typically used to improve performance [51–53]. In general, thermophilic digestion is more efficient due to a higher degradation rate that results in shorter retention time and increased methane production rate [24,54]. Furthermore, for feedstocks where pathogens are present, thermophilic temperatures can deactivate pathogens, eliminating the need for post-processing [40]. However, compared with mesophilic digestion, thermophilic digestion has higher energy requirements and needs careful process control to prevent process instability [55]. Process instability is of concern as thermophilic microorganisms are highly sensitive to temperature fluctuations [54,55] and higher temperatures can elevate ammonia levels [56]. Therefore, despite the potential for higher energy production from thermophilic digestion, trade-offs

include higher energy requirements and a lack of process robustness. For two-phase systems, it is also possible to operate each phase at different temperature regimes. The various combinations applied in LBR-AF experiments for different feedstock groups are presented in Tables 1 to 4. This section initially focusses on drawing knowledge from studies that directly compare different temperature regimes for particular feedstocks and operating conditions. Conclusions from these studies are then used to highlight potential research paths to gain a broader understanding of the influence of temperature on phase separation in LBR-AF systems.

Mesophilic (38 °C) and thermophilic (55 °C) AFs coupled to separate thermophilic LBRs has been compared for the digestion of rye

Table 1
Summary of process parameters, methane yield and methane content (if reported) for LBR-AF studies processing energy crops or crop wastes.

Feedstock (Co-substrate ratio, VS based)	Temj	perature	SRT	Filter Media	AF Flow Direction	Scale		Total CH <sub>4</sub> from LBR	CH <sub>4</sub> C	Content	CH <sub>4</sub> Yield	Ref.
	(°C)		(days)		(up/down)	(L)		(%)	(%)		(mL/gVS)	
	LBR	AF				LBR	AF		LBR	AF		
Peeled Potato	Mesc	philic	24	Pre-digested Straw	<b>↓</b>	10000	2600	10-30	-	60-78	567	[32]
Peeled Potato	Mesc	philic	24	Plastic	1	10000	2600	10-30	-	60–78	567	[32]
Potato	Mesc	philic	36	Pre-digested Straw	$\downarrow$	10000	2600	10-30	-	60–78	418	[32]
Potato	Mesc	philic	36	Plastic	1	10000	2600	10-30	-	60–78	418	[32]
Potato waste	37	37	38	Pre-digested Straw	1	2	1	-	0-56	69-82	390	[27]
Beet leaves+Potato (1:4.6)	Mesc	philic	21	Pre-digested Straw	$\downarrow$	10000	2600	10-30	-	60–78	650	[32]
Beet leaves+Potato (1:4.5)	Mesc	philic	21	Plastic	1	10000	2600	10-30	-	60-78	650	[32]
Beet leaves+Potato (1:3)	Meso	philic	21	Pre-digested Straw	$\downarrow$	10000	2600	10-30	-	60–78	647	[32]
Beet leaves+Potato (1:2.9)	Mesc	philic	21	Plastic	1	10000	2600	10-30	-	60-78	647	[32]
Beet leaves	Mesc	philic	21	Plastic	<b>↑</b>	10000	2600	>50	-	60-78	355	[32]
Beets	37	37	28	Plastic	<b>↑</b>	$0.8 \times 6$	0.9	_	-	-	440	[33]
Beets	37	37	55	Pre-digested Straw	<b>↓</b>	7600	2600	17	-	-	380	[34]
Beets	37	37	55	Plastic	<b>↑</b>	7600	2600	17	-	-	380	[34]
Grass/clover	37	37	50	Pre-digested Straw	$\downarrow$	7600	2600	36	_	_	390	[34]
Grass/clover	37	37	50	Plastic	<b>↑</b>	7600	2600	36	_	_	390	[34]
Grass/clover	37	37	26	Plastic	<b>↑</b>	$0.8 \times 6$	0.9	_	_	_	270	[33]
Grass	55	38	25	Polyethylene	<b>↑</b>	50	50	46	_	_	_	[25]
Grass+Maize+Rye (-)	55	38	25	Polyethylene	<u>†</u>	50	50	_	_	_	_	[31]
Grass+Maize+Rye (-)	55	38	21	Plastic	ļ	100	30	_	_	_	_	[31]
Grass	ta	t <sup>a</sup>	190	Inert Media	1	8000	190	_	_	71	_	[35]
Grass	ta	$t^{a}+5.5$	190	Inert Media	· 1	8000	190	_	_	71	_	[35]
Maize	35	35	28	Plastic	· 1	3.5	$4 \times 2$	41	_	_	434	[19]
Maize	35	35	28	Plastic	· 1	3.5	$4 \times 2$	38	_	_	433	[20]
Maize	35	35	7	Plastic	· 1	3.5	$1.5 \times 2^{b}$	70	_	_	422	[19]
Maize	35	35	7	Plastic	· †	3.5	$1.5 \times 2^{b}$	60	_	_	418	[20]
Maize	35	35	14	Plastic	· †	4	4	20–40	_	_	_	[36]
Maize	35	35	28	Plastic	· †	4	4	20-40	_	_	_	[36]
Maize	55	38	25	Polyethylene	· †	50	50	30	_	_	_	[25]
Maize	38	38	19–23	Polyethylene	i	220 × 2	12000 × 2	_	_	69–77	_	[37]
Maize	38	38	19-23	Polyethylene	į	$40000 \times 2$	$12000 \times 2$	_	_	69–77	_	[37]
Maize		philic	63	Plastic	<b>†</b>	10000	2600	_	_	_	_	[30]
Rye+Straw (–)	60	55	21	Plastic	i	100	30	_	56 Ov	erall	335	[38]
Rye+Straw (-)	55	55	21	Plastic	ì	100	30	_	51 Ov		314	[38]
Rye+Straw (–)	55	55	21	Plastic	ì	100	30	58	41	74	307	[29]
Rye+Straw (-)	65	55	21	Plastic	1	100	30	_	50 Ov		304	[38]
Rye+Straw (-)	70	55	21	Plastic	i i	100	30	_	50 Ov		258	[38]
Rye+Straw (–)	75	55	21	Plastic	1	100	30	_	65 Ov		247	[38]
Rye+Straw (–)	55	38	21	Plastic	¥ I	100	30	12	10	85	247	[29]
Rye	55	38	25	Polyethylene	<b>↓</b>	50	50	39	_	-	-	[25]
Willow Shoots	37	36 37	82 82	Pre-digested Straw	1	7600	2600	84	_	_	160	[34]
Willow Shoots	37	37	82 82	•	•	7600	2600	84	_	_		[34]
WIHOW SHOOTS	3/	3/	82	Plastic	1	/600	∠000	04	-	-	160	[34]

<sup>&</sup>lt;sup>a</sup>Ambient temperature.

Table 2
Summary of process parameters, methane yield and methane content (if reported) for LBR-AF studies processing OFMSW or MSW.

Feedstock (Feedstock: Inoculant, wet mass based)	Temperature (°C)		SRT	Filter Media	AF Flow Direction	Scale (L)		Total CH <sub>4</sub> from LBR	CH <sub>4</sub> Content (%)		CH <sub>4</sub> Yield	Ref.
			(days)		(up/down)			(%)			(mL/gVS)	
	LBR	AF	-			LBR	AF		LBR	AF		
Food & Yard Waste (1:1.5)	ta	35	16	Plastic	<b>↑</b>	28 × 3	114	_	_	_	262	[39]
Food & Yard Waste (1:0.11)	ta	35	16	Plastic	1	$28 \times 3$	114	_	-	-	232	[39]
Food & Yard Waste (1:0.67)	ta	35	16	Plastic	<b>↑</b>	$28 \times 3$	114	_	-	-	216	[39]
Food & Yard Waste (1:0)	ta	35	16	Plastic	<b>↑</b>	$28 \times 3$	114	_	-	-	211	[39]
Food & Yard Waste (1:0.25)	ta	35	16	Plastic	<b>↑</b>	$28 \times 3$	114	_	-	-	197	[39]
Paper, Food & Yard Waste (-)	37	37	60	-	_	4	12	_	_	72	197	[40]
Paper, Food & Yard Waste +	ta	ta	151	Plastic	<b>↑</b>	6400	$222 \times 2$	-	73 O	verall	190	[41]
Cattle Manure (-)												
Paper, Food & Yard Waste (-)	55	55	25-36	_	_	4	12	_	-	65	176	[40]
Paper, Food Waste, Plastic &	25	35	524	Plastic	<b>↑</b>	14	8	53	55	70-80	120	[42]
Textiles (-)												
Paper, Food & Yard Wastes, Plastic, Metal, Glass, etc. (–)	t <sup>a</sup>	t <sup>a</sup>	113	Plastic	1	6400	222 × 2	_	73 O	verall	30	[41]

<sup>&</sup>lt;sup>a</sup>Ambient temperature.

silage and barley straw [29]. For both configurations, hydrolysis was similarly efficient; however, methane yield and biogas quality varied significantly. For dual thermophilic reactors, higher methane yields were observed but biogas quality suffered due to poor phase separation.

The LBR produced 58% of the total methane yield with an average methane content of 41%, compared with an average methane content of 74% in the thermophilic AF. In contrast, the mesophilic AF produced 88% of the total methane yield with an average methane

<sup>&</sup>lt;sup>b</sup>Use parallel AF and upflow sludge blanket that perform similarly.

Summary of process parameters, methane yield and methane content (if reported) for LBR-AF studies processing manures.

Feedstock (Feedstock: Inoculant, wet mass based)	Temp	erature	SRT	Filter Media	AF Flow Direction	Scale		Total CH <sub>4</sub> from LBR	CH <sub>4</sub>	Content	CH <sub>4</sub> Yield	Ref.
	(°C)		(days)		(up/down)	(L)		(%)	(%)		(mL/gVS)	
	LBR	AF	-			LBR	AF		LBR	AF		
Cattle Manure + Cotton Gin	ta	t <sup>a</sup>	29	Plastic	1	6400	222 × 2	0	_	70	166	[43]
Waste + Grass (1:1.1:0.5)												
Cattle Manure + Cotton Gin	ta	ta	141	Plastic	<b>↑</b>	6400	$222 \times 2$	_	72 O	verall	100	[41]
Waste (1:0.9)												
Cattle Manure	ta	ta	73	Plastic	1	6400	$222 \times 2$	_	72 O	verall	80	[41]
Horse Manure	Meso	philic	36	Plastic	<b>↑</b>	10000	2600	100 <sup>b</sup>	-	-	-	[30]

<sup>&</sup>lt;sup>a</sup>Ambient temperature.

**Table 4**Summary of process parameters, methane yield and methane content (if reported) for LBR-AF studies processing aquatic weeds.

					Filter Media	ı								
Feedstock WRR <sup>b</sup>		Temperature		SRT	Туре	Surface Area	AF Flow Direction	Scale		Total CH <sub>4</sub> from LBR	CH <sub>4</sub> C	Content	CH <sub>4</sub> Yield	Ref.
	(mL/day)	(°C)		(days)		$(m^2/cm^3)$	(up/down)	(L)		(%)	(%)		(ml/gVS)	
		LBR	AF					LBR	AF		LBR	AF		
Macroalgae	100	ta	35	50	PVC Rings	0.24	<b>↑</b>	1.4	1.4	40	-	_	344	[44]
Macroalgae	100	ta	35	50	BFS <sup>c</sup>	5.45	<b>↑</b>	1.4	1.4	20	_	_	317	[44]
Macroalgae	200	ta	35	50	BFS <sup>c</sup>	5.45	<b>↑</b>	1.4	1.4	20	-	_	280	[44]
Macroalgae	200	ta	35	50	PVC Rings	0.24	<b>↑</b>	1.4	1.4	40	_	_	251	[44]
Macroalgae	50	ta	35	50	PVC Rings	0.24	<b>↑</b>	1.4	1.4	40	_	_	236	[44]
Macroalgae	150	ta	35	50	BFS <sup>c</sup>	5.45	<b>↑</b>	1.4	1.4	20	_	_	231	[44]
Macroalgae	50	ta	35	50	BFS <sup>c</sup>	5.45	<b>↑</b>	1.4	1.4	20	_	_	225	[44]
Macroalgae	150	ta	35	50	PVC Rings	0.24	<b>↑</b>	1.4	1.4	40	-	-	161	[44]
Ipomoea carnea	_	33	33	_	_	_	<b>↑</b>	0.9	0.9	_	-	70	_	[45]
Ipomoea carnea	_	33	33	_	-	-	<b>↑</b>	1.8	1.8	-	_	70	_	[45]

<sup>&</sup>lt;sup>a</sup>Ambient temperature.

content of 85%, but achieved 12% less of the methane potential. Despite sacrificing some methane potential, the use of the mesophilic regime for the AF enhanced phase separation and enabled production of higher calorific value biogas. The temperature difference between phases was proposed to inhibit methanogens in AF effluent, limiting leachate-based inoculation of the LBR (discussed further in Section 2.2) to help maintain phase separation [29]. Further research is needed to confirm this mechanism as no other studies compare a thermophilic LBR coupled to mesophilic and thermophilic AFs.

For the same feedstock, the coupling of an LBR at higher temperatures (55-75 °C) with a thermophilic (55 °C) AF has also been investigated [38,57]. The LBR temperature was incrementally increased by 5 °C from 55 to 75 °C, with each temperature held for three feedstock batches (21 day SRTs). For all temperatures below 65 °C, similar degradation rates, biogas yield and total methane content (~50%-55%) were observed. At 70 and 75 °C, degradation rates declined causing reductions in biogas yields of 29 and 38%, respectively. Interestingly, the methane content declined to 42% at 70 °C but increased to 65% at 75 °C. With the decline in performance there was a transition in the LBR bacterial community. At temperatures exceeding 65 °C, a reduction in prevalence of members of the Clostridiales order, and increases in members of the Bacteriodales and Thermotogales orders, resulted in decreased carbohydrate degrading potential and hence the overall degradation rate. Recirculation of effluent from the high temperature LBR was also found to influence methanogen communities in the AF with Methanobacteriales being prevalent at all conditions, but Methanosarcinales only becoming prevalent at higher temperatures. The use of different methanogenesis pathways could explain the variation in methane content at temperatures exceeding 65  $^{\circ}\text{C}.$  Overall, the results indicate that operating the LBR at temperatures exceeding 55 °C does not enhance LBR-AF performance.

In contrast to aforementioned studies, uniform application of mesophilic and thermophilic temperature regimes across both phases of an LBR-AF has been compared for the digestion of the organic fraction of municipal solid waste (OFMSW) [40]. Similar methane yields were observed for both regimes; however, the thermophilic regime halved the required SRT and reduced waste processing costs by deactivating pathogens. Similar to Schönberg and Linke [29], poor phase separation was observed for uniform regimes with the LBR accounting for approximately 60% of the total methane yield, with an average maximum methane content ranging from 49 to 57% across all experiments. In combination, these studies suggest that coupling a thermophilic LBR to a mesophilic AF may improve efficiency by reducing SRT and enhancing biogas quality. Reduced SRT is expected as a result of enhanced substrate degradation rate with increase in LBR temperature from mesophilic to thermophilic. However, the influence of temperature difference between reactors on phase separation requires further clarification. Of the temperature regimes applied in the reviewed literature, only 9% (6 tests) have applied thermophilic LBRs coupled to mesophilic AFs (Fig. 3), and these tests (see Table 1) only consider three energy crops: grass, maize and rye. This highlights the need for further research comparing other regimes with a thermophilic LBR coupled to a mesophilic AF to clarify if phase separation is improved for different feedstocks, and if so, determine the mechanisms resulting in enhancement. It should be noted that although pressure studies, summarised in Table 5 and discussed in Section 2.6, also consider thermophilic LBRs coupled to mesophilic AFs, they have not been included in Fig. 3 as compared to all other reviewed studies AF effluent is not recirculated back to the LBR.

Although coupling a thermophilic LBR to a mesophilic AF may improve efficiency and phase separation, application of thermophilic temperatures is not always feasible. 80% of LBR-AF studies have applied mesophilic and/or ambient conditions (Fig. 3), presumably due to lower energy requirements and stability concerns with thermophilic temperatures. 48% of the tests used uniform mesophilic temperatures,

<sup>&</sup>lt;sup>b</sup>Methane production occurs rapidly in the LBR so AF is never connected.

bWater replacement rate (WRR).

<sup>&</sup>lt;sup>c</sup>Blast furnace slag (BFS).

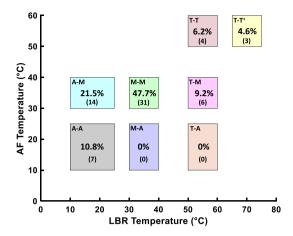


Fig. 3. Distribution of phase temperatures considered in LBR-AF studies. The boxes represent different temperature regimes and are labelled according to LBR, then AF, temperatures as: ambient (A), mesophilic (M), thermophilic (T) or hyperthermophilic (T+). The proportion and quantity of tests from LBR-AF studies (presented in Tables 1 to 4) that have used the temperature regime is indicated by the percentage and bracketed number in the boxes, respectively. Note that the boxes simply group studies and do indicate precise temperature ranges considered in reference to the axes.

21% used an ambient LBR coupled to a mesophilic AF, and 11% use only ambient conditions. There is potential to influence phase separation while applying lower temperatures through manipulation of other process parameters. For example, a mesophilic AF coupled to an ambient LBR (no temperature control, range not indicated) for the digestion of macroalgae observed both successful and unsuccessful phase separation depending on the type of filter media used [44] (discussed in Section 2.7). Additionally, limited methane production in the LBR has been observed for cattle manure co-digestion at ambient temperatures (>23°C) [43]. These studies highlight that the use of mesophilic or potentially psychrophilic (<20 °C) temperature regimes, in conjunction with other process parameter alterations, may enable successful phase separation. This provides an opportunity for further research considering less energy-intensive temperature regimes to understand the interplay between phase separation, temperature and other process parameters.

# 2.2. AF effluent recirculation and recycling inoculum

Two interrelated operational factors that influence the level of phase separation in LBR-AF systems are: (i) if leachate that has passed through the AF is recirculated back to the LBR, and (ii) if digestate and leachate are recycled as inoculum. Practical considerations such as minimising water consumption and waste management make leachate recirculation and recycling of digestate and leachate preferable. This section considers the role of these practices, initially recirculation alone, then both combined, on LBR-AF phase separation and performance.

LBR-AF systems can be operated with or without recirculation of leachate from the AF back to the LBR. A direct comparison of the two strategies has been made for the digestion of maize - a rapidly degrading substrate [36]. Recirculation of AF effluent stabilised LBR pH to promote hydrolysis. Two mechanisms were proposed for buffering of the LBR via leachate recirculation: (i) abiotically as a buffer solution (AF removes VFAs from leachate), and (ii) biotically through inoculation to enhance VFA removal in the LBR. Elevated LBR pH due to abiotic buffering by the AF would also promote leachate-based inoculation. This combined effect may explain contrasting observations for cattle manure digestion where minimal microorganism transfer between leachate and feedstock was observed in a single-phase LBR [58], but leachate-based inoculation occurred in an LBR-AF system [41,43,59]. Although this buffering effect is beneficial for substrates with rapid

pH decline, for slowly-degrading substrates buffering can instead limit hydrolysis by promoting pH levels optimal for methane production, and consequently poor phase separation. The suitability of such substrates for LBR-AF systems is discussed further in Section 3. It should be noted that all studies summarised in Tables 1 to 4 recirculated AF effluent.

Although recirculation has been shown to mitigate VFA accumulation, it can also promote the accumulation of process inhibiting substances that can adversely affect methane production and digestate and leachate quality. Depending on feedstock characteristics, key inhibitors that may be present in systems that recirculate process liquid include ammonia, heavy metals, salinity and sulfides [64]. Elevated levels of ammonia and salinity have been observed for OFMSW digestion [39], and high sulfide levels for macroalgae digestion [44], in LBR-AF systems. Similarly, recirculation results in elevated ammonia [65,66] and sulfide levels [65] for poultry manure in single-phase LBRs. Additionally, for digestion of various energy crops in LBR-AF systems, mobilisation of heavy metals in leachate occurs at low pH and is therefore promoted by improved hydrolysis and ongoing acid production [30,34]. Similar observations have been made for bioreactors digesting MSW with heavy metal dissolution in leachate corresponding to high levels of VFA production [67]. These examples highlight key inhibitors that may be present in recirculating systems depending on feedstock characteristics. Therefore, for certain feedstocks, there is a need to understand how recirculation influences inhibitor levels in LBR-AF systems and the impact on methane production and digestate and leachate quality.

Recirculation influences phase separation and inhibitor levels in LBR-AF systems; however, there is limited published understanding on how different recirculation parameters influence LBR-AF performance. Recirculation parameters include pumping rate and frequency (continuous or intermittent) between reactors, and if applicable for separate recirculation in the individual reactors. Reporting of these recirculation parameters in the reviewed literature is inconsistent and often lacking justification for the chosen recirculation strategy. Furthermore, no study was identified that compares the influence of different recirculation parameters in LBR-AF systems. This lack of knowledge on the influence of recirculation parameters on phase separation, inhibitor levels and methane yield in LBR-AF systems constitutes a clear research gap that needs to be addressed. Standardised reporting of recirculation parameters would also assist with assessment and comparison of literature. Further discussion on findings for recirculation parameters in single-phase LBRs and potential implications on LBR-AF performance is provided in Section 4.3. Although the influence of recirculation parameters on LBR-AF performance has not been considered, the impact of recycling digestate and leachate in long-term operation has been investigated.

Long-term LBR-AF studies using leachate recirculation and recycling digestate and leachate as inoculum have been performed for the digestion of maize silage [19,20] and OFMSW (food and yard wastes) [39]. For maize silage digestion, all digestate and inoculum were recycled from preceding batches which resulted in LBRs transitioning to methanogenic reactors after several feedstock batches [19, 20]. Leachate-based inoculation was evident with leachate containing similar numbers of methanogens and greater numbers of hydrolytic microorganisms than feedstock and digestate by the end of experiment [19]. For maize digestion, accumulation of inhibitors due to recycling digestate and leachate was not reported; however, this is a common concern for feedstocks such as food wastes [39]. Consequently, it has been investigated if varying the feedstock-to-digestate ratio (with all leachate recycled) can help overcome elevated ammonia and salinity levels [39]. Feedstock-to-digestate ratios ranging from 1:1.5 to 1:0 (wet weight basis) were investigated. At start-up or when inhibitor levels rise, higher feedstock-to-digestate ratios between 1:0.67 and 1:1.5 were required to accumulate inhibitor-tolerant microorganisms in the leachate. Once accumulated (within two to four batches), a lower feedstock-to-digestate ratio of 1:0.11 resulted in improved

Table 5
Summary of parameters from studies coupling thermophilic LBRs to pressurised, mesophilic, upflow AFs.

			Filter Media			Anaerobic Filte	er		
Feedstock	Pressure (MPa)	RF <sup>a</sup> (L/d)	Туре	Surface Area (m²/m³)	Porosity (%)	CH <sub>4</sub> content (%)	CH <sub>4</sub> yield (mL/gCOD)	Ref.	
Maize	0.1	_	Sintered Glass	270000	70	66.4	330	[60]	
Maize	0.1	_	Sintered Glass	270000	70	67.0	333	[61]	
Maize+Grass	0.1	-	Sintered Glass	270000	70	71.0	304	[61]	
Maize	0.3	-	Sintered Glass	270000	70	69.5	330	[60]	
Maize	0.3	-	Sintered Glass	270000	70	70.0	331	[61]	
Maize+Grass	0.3	_	Sintered Glass	270000	70	71.0	288	[61]	
Maize	0.6	_	Sintered Glass	270000	70	71.8	330	[60]	
Maize	0.6	_	Sintered Glass	270000	70	72.0	326	[61]	
Maize+Grass	0.6	-	Sintered Glass	270000	70	73.0	285	[61]	
Maize	0.9	-	Sintered Glass	270000	70	75.6	310	[60]	
Maize	0.9	_	Sintered Glass	270000	70	76.0	313	[61]	
Maize	0.9	0	Sintered Glass	270000	70	75	320	[62]	
Maize	0.9	20	Sintered Glass	270000	70	82.0	320	[62]	
Maize	0.9	40	Sintered Glass	270000	70	87.0	320	[62]	
Maize+Grass	0.9	_	Sintered Glass	270000	70	77.0	258	[61]	
Maize+Grass	1.0	-	Polyethylene	861	83	79.0	330	[63]	
Maize+Grass	2.5	_	Polyethylene	861	83	87.0	313	[63]	
Maize+Grass	5.0	-	Polyethylene	861	83	90.0	260	[63]	

<sup>&</sup>lt;sup>a</sup>Recycled flow (RF) rate of effluent circulated through flash tank to scrub CO<sub>2</sub> then re-pressurised and recirculated.

hydrolysis and methane yield. As different hydrolytic microorganisms were dominant in digestate and leachate, they likely complement each other as inocula [39]. Overall, these studies indicate that leachate recirculation and recycling of digestate and leachate can enhance hydrolysis through buffering and leachate-based inoculation with microorganisms acclimated to the presence of inhibitors. However, this practice also transitions LBRs to methanogenic reactors over time, which can limit substrate degradation and acid production. This detrimental effect on phase separation limits the benefit of using a methanogenic reactor for higher-quality biogas production.

The studies considered in this section have demonstrated that leachate-based inoculation occurs due to leachate recirculation and recycling of digestate and leachate, and that this leads to poor phase separation in LBR-AF systems. Further research is needed to better understand leachate-based inoculation. For example, it is likely that the effects vary based on feedstock characteristics. With further research and understanding of leachate-based inoculation, a strategy could be developed to maintain phase separation and optimise LBR-AF performance. A strategy for helping to maintain phase separation could be the use of pH control to maintain LBR pH at a level inhibitive to methanogenesis and conducive to hydrolytic microorganisms. The control of pH through recirculation without chemical additives has been shown to enhance performance for continuous stirred-tank reactors coupled with AFs [68,69]. A similar technique could be investigated for an LBR-AF system. Further investigation of recirculation parameters may enable development of such a method. In summary, there are aspects of leachate recirculation, as well as inoculation strategy, that need further research in order to enhance and maintain phase separation for long-term operation of LBR-AF systems.

#### 2.3. Solid retention time

Long-term operation of LBR-AF systems is influenced by the frequency of feedstock replacement for the period of operation (number of feed cycles) and thus the duration that feedstock is digested before replacement (solid retention time). This section considers studies that have compared different SRTs for the same feedstock and operating conditions. SRTs of 7, 14 and 28 days have been compared for mesophilic digestion of maize silage [19,20,36]. The shortest duration achieved higher daily degradation rates and acid and methane production; however, the shorter degradation time resulted in lower specific methane yields (methane per mass of volatile solids, VS) and increased suspended solids in the leachate. Longer retention times were considered favourable due to increased solubilisation of solids

in the leachate [19]. For the longer durations, the 14-day cycle was suggested over the 28-day cycle as similar specific methane yields were observed but the average daily methane production rate was higher. In terms of phase separation, for all SRTs, the recycling of all digestate and leachate from preceding batches led to poor phase separation over time, as discussed in Section 2.2. Therefore, it may be necessary to consider the combined effects of SRT and inoculation strategy to optimise phase separation and methane yield for long-term operation of LBR-AF systems.

For maize digestion, these studies provide an indication of the influence of SRT on methane yield and suspended solids in leachate. However, detailed information on the composition of digestate and leachate is lacking. Anaerobic digestion changes the availability of macro- and micronutrients in digestate and leachate compared to feed-stock [70]. Different levels of substrate degradation with varying SRTs may influence the availability, form and distribution of nutrients in digestate and leachate. The quality of digestate and leachate is an important practical consideration that influences applicability as fertiliser (potentially a value-adding product) or requirements for waste disposal (a cost). Therefore, further research should be conducted for different feedstocks to understand the influence of SRT on both methane yield, and digestate and leachate quality. Other practical factors that may also be important to consider regarding SRT are feedstock availability and handling costs, as well as odour control for feedstocks such as manure.

# 2.4. Flow mode and hydraulic retention time

The typical modes that anaerobic filters can be operated in are upflow, submerged downflow or trickling downflow (non-submerged or partially submerged). The submerged downflow and upflow modes operate similarly by completely submerging the filter media. However, for downflow reactors, microorganism growth is likely to be highest towards the top of the filter and liquid mixing is enhanced by concurrent gas flow, and vice versa for upflow [71]. In contrast to these modes, trickling downflow operates like an LBR in that leachate percolates through unsubmerged filter media. As trickling systems have low hydraulic retention time, recirculation through the AF is often required [72]. This section will discuss comparisons between AF flow modes that have been used in studies employing LBR-AF systems.

Upflow and submerged downflow AFs coupled to an LBR have been compared for the digestion of potato waste and sugar beet leaves [32]. No significant difference in performance was observed; however, this was not a direct comparison as different filter media (straw and plastic) were used for the different flow directions. In contrast, different flow

modes have been observed to significantly influence phase separation for the co-digestion of rye, grass and corn [31]. The fraction of methanogenesis in the LBR for the system with an upflow AF was approximately 46% compared with 11% for the downflow AF. Despite this difference, the distribution of hydrolysis, acidogenesis and acetogenesis were similar for both systems. The factor influencing the distribution of methanogenesis was the HRTs of the downflow and upflow AFs of 1.2 and 12.8 days, respectively. Regardless, these studies indicate that flow direction has minimal impact and rather HRT is a key influence on the distribution of methanogenesis in an LBR-AF system. Further research is needed to better understand the influence of AF HRT on phase separation and the performance of LBR-AF systems.

The majority of literature using LBR-AF systems use upflow AFs (see Tables 1 to 5). As HRT appears to have significant influence and there is little difference between submerged flow modes, perhaps trickling downflow anaerobic filters with low HRT should be investigated. However, it should be noted that low HRTs (less than 1 day) have been suggested as a method for suppressing methane production in single-phase AFs [73]. Therefore, investigation of submerged systems with control of HRT may be more insightful. Finally, as for recirculation parameters, it should be noted that reporting on HRTs is inconsistent and that standardised reporting would assist with assessment of the literature.

#### 2.5. Organic loading rate

Studies have considered isolated AFs digesting leachate with high VFA concentrations to systematically study the influence of OLR [16, 74]. Despite not considering the coupled system, these studies provide insight into the stability of the AF as OLR varies, as well as the maximum OLR before process failure, thereby providing a basis for LBR-AF system design. For reference, OLR is typically measured on either a COD or VS basis. While COD indicates the amount of organic matter present based on the oxygen that is required to oxidise the substrate, VS content indicates the amount of organic matter present based on mass loss during substrate ignition. The VS content may also be adjusted to account for the loss of VFAs during the drying phase before substrate ignition.

For the digestion of potato waste leachate, OLR was varied between 1.5 and 7 g COD/L/d [16]. The AF adapted rapidly to sudden changes in OLR and operated stably to a maximum OLR of 4.7 g COD/L/d. As the OLR increased to 4.7 g COD/L/d, the specific methane yield increased and methane content decreased (80 to 66%), indicating that methanogenesis becomes rate-limiting. Above the maximum OLR, rapid pH reduction due to VFA accumulation, and possibly clogging of the filter resulting in channelling, resulted in decreased methane yields. In contrast, stable operation and a decreasing methane yield trend with increasing OLR from 2.4 to 25 g COD/L/d was observed for the digestion of synthetic energy crop leachate [74]. Although further research is required to better understand the differences in maximum OLR for the different feedstocks, these studies indicate that AFs should be able to rapidly adapt to changing VFA production from coupled LBRs. As the maximum OLR would be dependent on factors such as AF size, filter medium properties (discussed in Section 2.7) and reactor start-up (i.e., microbial-community development), research would need to consider the interplay between such factors to adequately assess the maximum OLR of an AF.

#### 2.6. Pressure

Pressure is not generally considered a critical parameter for improving anaerobic digestion. However, several studies (summarised in Table 5) have considered LBRs coupled to pressurised AFs with the aim of achieving production of near pipeline-quality (>95% methane) biogas [60–63]. Furthermore, as the technology combines biogas production, upgrading and pressurisation, it has potential to be a viable

alternative for production of pipeline-quality biogas [63]. Similar to biogas upgrading technologies (e.g., water washing), the premise for increasing AF pressure is that carbon dioxide more readily dissolves in water than methane. Consequently, increasing pressure could further enhance the methane content of biogas produced in the methanogenic reactor of a phase-separated system [60]. However, as these pressure studies do not recirculate AF effluent to the LBR, no indication of the effect of pressure on phase separation is provided. This section discusses the influence of increasing pressure on AF methane yield and content, and potential effects this could have on an LBR-AF system recirculating AF effluent.

Pressurising the AF in an LBR-AF system was initially investigated for the digestion of maize silage [60]. Elevating pressure from 0.1 to 0.9 MPa increased methane content from 65 to 75%; however, a 24% drop in biogas yield resulted in a slight decrease in methane yield. The decreased methane yield and lack of pipeline-quality biogas were attributed to the AF pH declining from 7.2 to 6.5. A decline in pH is expected as dissolved carbon dioxide forms carbonic acid that releases protons upon dissociation. This lower pH reduces the solubility of carbon dioxide [60] and is sub-optimal for methanogens [23]. The same pressures were applied for co-digestion of grass and maize silages to investigate if increasing ammonium concentrations could buffer the system [61]. The increase in pH enhanced methane content slightly at all pressures, but decreases in methane yields were observed compared with the unbuffered system. Increased methane solubility due to higher ammonium concentrations was proposed to explain decreased methane yields [61]. For this reason, while investigating pressures between 1 and 5 MPa, methane solubility was accounted for by releasing dissolved methane in flash tanks [63]. Elevating pressure from 1 to 5 MPa increased methane content from 79 to 90%; however, despite accounting for dissolved methane, there was a 12% decrease in methane yield. Observed propionic acid accumulation was cited to explain the decreased methane yields [63], as high carbon dioxide partial pressures can inhibit propionic acid conversion [75]. Although ammonium nitrogen concentrations detected in the system were reported to be below inhibitory concentrations [60], the use of other pH control methods would rule out ammonia inhibition as a potential cause of methane yield decline. A flash tank has also been trialled for maize digestion at 0.9 MPa to scrub carbon dioxide from effluent before repressurisation and recirculation through the AF [62]. With increasing recirculation rate of scrubbed effluent to a maximum of 40 L/d, pH increased from 6.5 to 6.7 and methane content increased from 75 to 87%, without a decline in methane yield. However, a significant proportion of methane (up to 25%) was released in the flash tank. This is counter-productive as a key motivation for pressurised AFs is the ability to combine biogas production, upgrading and pressurisation (autogeneratively via gas build-up to desired pressure) in one step [62]. Therefore, other methods to counter pH decline in pressurised AFs should be investigated.

The decline in AF pH and methane yield with increasing pressure may also be of concern in an LBR-AF system recirculating AF effluent. If the LBR is operated at atmospheric pressure, it would act similarly to a flash tank with carbon dioxide being released from effluent, although more effective as acidic conditions reduce carbon dioxide solubility [62]. Furthermore, as the effluent is more acidic than expected at atmospheric pressure, it is plausible that increased AF pressure may slow buffering and transition of the LBR to a methanogenic reactor; consequently, enhancing substrate degradation and VFA production. Enhanced AF methane content is an additional benefit. However, suboptimal pH and potential inhibition of methanogens in the AF may promote accumulation of VFAs and limit the yield of methane-rich biogas. Therefore, although increasing AF pressure may have some stimulatory effects, inhibitory effects on AF performance potentially outweigh the benefits. Research is required to elucidate the effects of pressure on LBR-AF systems recirculating effluent.

Despite not achieving pipeline-quality gas, pressurising an AF that is fed with leachate from an LBR can significantly enhance biogas quality. However, the high investment cost to pressurise reactors and technical challenges such as pH decline are drawbacks to this method [76]. As the technology combines biogas production, upgrading and pressurisation, with further development it may become a viable alternative for production of pipeline-quality biogas. Although this technology may reduce the required upgrading [60,63], it could be necessary to further consider removal of trace impurities such as water vapour, hydrogen sulfide and siloxanes. Furthermore, separation and purification of carbon dioxide is not considered. Producing food-grade carbon dioxide in addition to pipeline-quality biogas provides further economical benefit and limits carbon dioxide release into the atmosphere [77]. As pH issues were related to dissolved carbon dioxide in leachate, perhaps separation of carbon dioxide may also assist with pH control.

#### 2.7. Filter media

The range of different filter media types used in studies employing an LBR-AF system include blast furnace slag, glass, straw and various forms of plastic, such as tower packing or polyethylene fillers (Tables 1 to 5). Most studies use some form of plastic, but a limited number have compared the use of different filter media types. This section will discuss the findings of these studies and the lack of focus on the influence of media properties on LBR-AF system performance.

Straw has been compared with other common filter media as it is an abundant and inexpensive material. Straw was found to be superior to glass at higher OLRs as it maintained higher total VFA degradation rates [74]. In addition to serving as a filter, straw also contributes to methane yield as it is biodegradable. This resulted in overestimation of methane yields at low OLRs, but was considered negligible at high OLRs [74]. The methane contribution of straw was limited in further studies by using pre-digested straw [32,34]. As straw biodegrades, albeit slowly, the structural stability of straw for long term use is also questionable. Plastic was observed to outperform pre-digested straw that had been in use for two years prior to the experiment [34]. It was suggested that structural breakdown of the straw may have caused channelling and consequential decrease in performance. Conversely, straw that had been pre-digested for 14 months prior to the experiment was found to be structurally stable and perform similarly to plastic [32]. This suggests that straw may be viable for use for 1.5 to 2 years before needing replacement. Each replacement requires a lengthy start-up period to develop microbial communities, likely negating the cost benefits of using the cheaper material. Therefore, these issues likely make straw unsuitable for LBR-AF systems despite potential performance enhancement.

Blast furnace slag and PVC rings have also been compared as filter media while investigating digestion of macroalgae at various water replacement rates (replacing AF effluent with water) [44]. While poor phase separation occurred when using plastic, blast furnace slag resulted in 78%-83% of the methane production occurring in the AF. It was suggested that the significantly higher specific surface area of the blast furnace slag (2.37 m<sup>2</sup>/g compared with 0.16 m<sup>2</sup>/g) enhanced methanogen retention, reducing washout and transfer to the LBR. However, other properties such as surface roughness and porosity likely influence performance. A shift in the archaeal community in the LBR towards that of the plastic packed AF supported that methanogen retention was better in the filter with blast furnace slag. This suggests that filter media properties can significantly influence phase separation. As macroalgae is sulfur rich [44], inhibition due to sulfide and the production of hydrogen sulfide is also a concern. Interestingly, at higher water replacement rates, the PVC rings reduced hydrogen sulfide production by 45 to 53%. The cause of this reduction is unclear but it does demonstrate that filter media type may influence inhibitor levels in LBR-AF systems and can impact biogas quality in terms of reducing hydrogen sulfide production.

In pressure studies (Section 2.6, Table 5), different filter media have also been used for digestion of maize and grass at AF pressures of 0.9

and 1 MPa, respectively [61,63]. The use of polyethylene fillers (surface area of 861  $\rm m^2/m^3$  and porosity of 83%) at 1 MPa [63] resulted in a 28% increase in specific methane yield compared to sintered glass (effective settlement area of 270000  $\rm m^2/m^3$  and porosity of 70%) at 0.9 MPa [61]. As increasing pressure has been observed to decrease methane yield [60,61,63], this suggests that differences in methane production may have been due to filter media type. The higher methane yield was achieved for media with larger porosity and significantly lower surface area; though the surface area of sintered glass accessible to microorganisms may be significantly less than the reported value. As these studies do not recirculate AF effluent to the LBR, no indication is provided on phase separation. Regardless, the increase in methane yield highlights the need for further research considering filter media properties such as porosity.

Despite evidence that filter media properties influence LBR-AF system performance, there has been limited investigation of different properties in LBR-AF studies. From low-solids digestion studies there is some understanding of the influence of different media properties on AF performance. The performance of AFs depends mostly on the ability to retain microorganisms and distribute flow throughout the filter (i.e., not channel). Porosity and surface roughness have been suggested as the most important factors for developing biofilms and minimising microorganism washout [78-82]. Specific surface area is typically considered less important than these properties [78,79,83]; however, significant performance improvement with increasing surface area has also been reported [84]. In terms of flow distribution, the pore size, media geometry and stacking method (i.e., some media can be stacked to induce cross-flow) have been shown to influence the level of lateral flow, and thereby reduce channelling, which subsequently improves performance [79,80]. Related to this, mechanical resistance of the media is also considered important to prevent channelling [78,81], but this is typically not assessed due to the time required for assessment. This information from low-solids digestion studies provides an indirect guide regarding the influence that different properties may have on an LBR-AF system. However, direct investigation of properties in a coupled LBR-AF system is required to fully understand the influence of filter medium properties on phase separation and performance.

The few studies that have compared different filter media in LBR-AF systems indicate that the use of different filter types may significantly influence phase separation and methane yield. This highlights the need for further research comparing different filter media types and properties to elucidate the effects on LBR-AF performance. It is also evident from review of the literature that the potential of filter media to reduce the impact of inhibiting substances in LBR-AF systems has had minimal consideration. In addition to promoting microorganism attachment and biofilm formation, the use of adsorbent materials (e.g., biochar) may reduce inhibiting substances in leachate. This subject is addressed further in Section 5. Finally, it should be noted that current literature typically lacks information on filter media properties and that standardised reporting could enable better comparison of results between different studies.

#### 3. Feedstock type and co-digestion

Studies employing LBR-AF systems have primarily investigated energy crops or crop residues, but municipal solid waste, manure and aquatic plants or macroalgae have also been considered (Tables 1 to 4). For some feedstocks, co-digestion with different substrates has been investigated. The primary reason for co-digestion is to balance the carbon-to-nitrogen ratio (C/N) (ideally between 20:1 and 30:1) in terms of microorganism requirements for growth and energy [52]. Other reasons may include increasing buffering capacity [54,61], improving moisture content [52], ensuring a more complete trace element profile [85], decreasing the influence of inhibitory substances, and increasing microbial community diversity for enhancement of hydrolysis [52,54]. The following subsections will present key findings for the feedstocks that have been investigated in an LBR-AF system, and where applicable the influence of co-digestion, with particular focus on phase separation.

#### 3.1. Crop residues and energy crops

Mono-digestion of various crop residues or energy crops have been directly compared in LBR-AF systems. For digestion of maize, rve and grass silages, the LBR contributed 30, 39 and 46% of the methane yield, respectively [25]. The better phase separation for maize and rye was attributed to longer ongoing acid production (~10 days) that maintains a pH suitable for hydrolysis, but inhibitive to methane production in the LBR. Similarly, the biodegradability characteristics of willow shoots, sugar beets and grass influence phase separation [34]. For these substrates, 84, 17 and 36% of the methane yield was produced in the LBR, respectively. The biodegradability of willow shoots was low due to high lignin content, resulting in low VFA production. This produced pH conditions in the LBR suitable for methane production and thus operation as a single-phase LBR. Conversely, rapid biodegradation of sugar beets and grass produced pH conditions that improved phase separation in the LBR-AF system. For these two feedstocks, after hydrolysis becomes rate-limiting (declining VFA production and increasing pH), a shift in LBR microorganism population has been observed with methanogens becoming more prevalent and bacteria decreasing [33]. Therefore, replacement of feedstock before VFA production becomes too low and poor phase separation occurs may be prudent for production of higher quality biogas. For example, for these feedstocks it was observed that majority (~85%) of the methane yield was produced within 30 days, mostly in the AF (97 and 77%, respectively) [34]. Perhaps biological or chemical process variable monitoring could indicate suitable timing for feedstock replacement. Monitoring substrate-specific enzyme activity as an indicator of hydrolysis progress has been recommended as a quick and inexpensive method compared with analysing chemical variables (e.g., VFA to total alkalinity ratio) [37]. Overall, combining prudent batch durations with a feedstock that suitably degrades to maintain LBR pH conducive to hydrolytic bacteria, but inhibitive to methanogens, may promote phase separation and thereby enable production of higher quality biogas.

A limited number of studies have also compared mono- and codigestion of energy crops or crop residues. Compared with potato waste mono-digestion, a 60% increase in methane yield has been observed when co-digesting potato waste and sugar beet leaves (2:1 and 3:1 wet weight ratios) [32]. This improvement was attributed to the nitrogenrich sugar beet leaves improving nutrient balance and buffering the LBR [32]. The buffering provided a more suitable pH for hydrolysis, resulting in a higher OLR for the AF. In terms of phase separation, both mono-digestion of potato wastes and co-digestion with sugar beet leaves limited methane production in the LBR to 10 to 30% of the methane yield. In contrast, for mono-digestion of sugar beet leaves the LBR rapidly became methanogenic. This demonstrates that co-digesting a poorly degradable substrate such as sugar beet leaves, with a rapidly degrading substrate such as potato wastes, may achieve the required level of VFA production to maintain suitable pH in the LBR to improve phase separation.

Co-digestion of maize and grass has also been compared with monodigestion of maize [61]. This co-digestion was investigated to increase ammonium concentrations to study the influence of buffering on high pressure AF conditions (discussed in Section 2.6). As ammonia is a process inhibitor, this highlights the importance of considering the nitrogen content of substrates and C/N of mixtures when selecting cosubstrates for co-digestion in LBR-AF systems. Other studies have also used co-digestion but have not directly compared with mono-digestion, as other process parameters such as pressure [63] or temperature [29, 38] were the focus. The use of different process parameters makes comparison between different studies challenging, and further research into the influence of co-digestion of different energy crops or crop residues on phase separation is required. It is notable that different co-substrate mixing ratios are rarely considered in co-digestion studies. Therefore, both different feedstock types and co-substrate mixing ratios should be investigated in future co-digestion research for LBR-AF systems.

The key findings from review of LBR-AF studies digesting crop residues and energy crops are: (i) slowly degrading feedstocks are more suited to single-phase LBRs; and (ii) rapidly degrading substrates with ongoing acid production that maintains pH suitable for hydrolysis, but inhibits methane production in the LBR, are suited for phase separation in an LBR-AF system. Furthermore, co-digestion of poorly and rapidly degrading substrates can potentially promote both rapid and ongoing VFA production suitable for phase separation in LBR-AF systems. Despite crop residues and energy crops getting considerably more attention than other feedstock types for LBR-AF systems (see Tables 1 to 4), there are still a limited number of studies that have focussed on co-digestion. Further research considering co-digestion of substrates with differing degradation characteristics could provide further insight into suitable feedstock characteristics for phase separation in LBR-AF systems. As rapid degradation and ongoing VFA production has been observed to provide suitable pH control for phase separation, other parameters that influence hydrolysis and pH regulation should also be considered. This further highlights the need for investigation of leachate recirculation strategies and the effect on pH control in LBR-AF systems. Section 4 aims to draw knowledge from key studies looking to optimise leachate recirculation in single-phase LBRs.

#### 3.2. Municipal solid waste

Municipal solid waste (MSW) is a complex feedstock that is composed of both biodegradable organic substrates (food, paper and yard wastes) and nondegradable inorganic materials (plastics, metals, glass, rubber, textiles, etc.). An LBR-AF system has been investigated for the digestion of mostly organic simulated MSW (71% fruit and vegetables, 13% paper, 9% plastic and 7% textiles, on a dry weight basis) [42, 86]. Initial operation as a single-phase LBR (152 days) lead to VFA accumulation and a pH decline that inhibited hydrolysis, acidification and methanogenesis in the LBR. Following this period, leachate was recycled once per day through the AF and recirculated back to the LBR. Leachate recycle rates of 128, 256 and 384 mL/day (AF HRTs of 62.5, 31.3 and 20.8 days, respectively) were investigated consecutively for periods of 57, 39 and 87 days, respectively. The initial period of leachate recirculation at 128 mL/day alleviated inhibition of hydrolysis and acidification in the LBR, and produced biogas with 70 to 80% methane content in the AF. Increasing the leachate recycle rate above 128 mL/day increased pH in the LBR and transitioned it to a methanogenic reactor. As the LBR utilised VFAs to produce lower quality biogas (20 to 55% methane content), the AF OLR declined. Consequently, the production rate of higher quality biogas in the AF declined to the point where it was unnecessary to operate the system in two phases. Overall, this study indicates that for MSW, tailoring of the leachate recirculation rate may improve or maintain phase separation. This further highlights the need for investigation of leachate recirculation strategies in LBR-AF systems.

As discussed in Section 2.2, there is a lack of understanding on the influence of recirculation on inhibitor levels in LBR-AF systems. This is particularly concerning for treatment of MSW and OFMSW as ammonia, salinity [39] and heavy metals [67] are typically present. Re-use of acclimated digestate and variation of the feedstock-to-digestate ratio has been shown to enhance hydrolysis, albeit leading to poor phase separation, for long-term digestion of OFMSW in the presence of inhibitors (discussed in Section 2.2). However, other methods may be able to mitigate inhibitor levels in LBR-AF systems. For example, further research could consider mono-digestion of OFMSW with co-digestion with other substrates. Balancing the C/N may reduce ammonia levels, in addition to the aforementioned benefits of co-digestion. The use of an absorbent filter medium, as discussed in Sections 5 and 2.7, could also reduce heavy metal and salt concentrations in leachate. These are just a few potential strategies that could be considered for mitigation of inhibitors in LBR-AF systems. Research investigating the influence of recirculation parameters on inhibitor levels, and the effect of potential mitigation

strategies, is needed to assess the capability of LBR-AF systems for treatment of substrates such as OFMSW where inhibitors are a concern. Overall few studies have considered MSW and OFMSW in LBR-AF systems ( Table 2), indicating the need for more research.

#### 3.3. Manure

LBR-AF systems have been considered for the digestion of cattle and horse manures. Single-phase LBRs have been recommended for horse manure as the slow degradation rate and thus limited pH decline promotes methanogenesis in the LBR within 4 days [30]. In contrast, for cattle manure digestion in a single-phase LBR, observed rapid accumulation of VFAs in leachate indicates potential for enhanced biogas production in two-phase systems [87]. This section considers findings on phase separation for cattle manure digestion in LBR-AF systems and considers LBR-AF application for other livestock manures.

An LBR coupled to two AFs has been investigated for co-digestion of cattle manure and cotton gin waste [41,59]. Rapid VFA production and pH decline delayed methanogenesis in the LBR and enabled immediate production of methane-rich (73%-86% methane) biogas in the AFs. However, within 15 days a rise in pH promoted rapid transition of the LBR to a methanogenic reactor. Leachate circulation to the AFs was terminated after 45 days as VFA concentrations in leachate became negligible. At this stage, 80% of the biogas yield had been recovered; 74% of this produced in the LBR with an average methane content of 62%, and 26% produced in the AFs with an average methane content of 79%. Despite the initial suppression of methanogenesis in the LBR, poor phase separation limited the effectiveness of coupled AFs for cattle manure and cotton gin waste co-digestion. In a further study, co-digestion of cattle manure, cotton gin waste and grass was investigated [43]. The combination of these substrates maintained pH at a level that suppressed methanogenesis in the LBR until near the experiment end (29 days) when minimal VFA production was observed. This suggests that digesting cattle manure with co-substrates with higher biodegradability may enhance LBR-AF process efficiency by shortening SRT and suppressing methanogenesis in the LBR to enhance phase separation. Further research should consider co-digestion of cattle manure with co-substrates with varying degrees of biodegradability to further understand the influence on LBR-AF phase separation and process efficiency. Regardless of the degree of phase separation, these studies have observed higher quality biogas than typically observed for high-solids systems co-digesting cattle manure and crop residues. For such systems, average methane contents of approximately 55% [4,88] are commonly reported.

Other common livestock manures (swine and poultry) with higher methane potentials [89] have not been considered for LBR-AF systems despite the benefits observed for cattle manure digestion. This is presumably due to concerns about high concentrations of ammonia that can be formed during degradation of these higher nitrogen content manures [64] and issues with poor porosity limiting leachate percolation (discussed in Section 4.1). Concerns of ammonia inhibition are evident for poultry manure with single-phase LBR studies considering strategies for ammonia removal [66,90]. Whereas, for swine manure digestion, only low ammonia levels have been observed in an LBR coupled to a continuous stirred-tank reactor [91,92]. Anaerobic digestion of poultry and swine manures could be considered in LBR-AF systems. Compared to single-phase LBRs, the use of an AF for biofilm development and tailoring of environmental conditions would be expected to enhance methanogen resistance to ammonia. Furthermore, as discussed for MSW in Section 3.2, co-digestion to balance the C/N ratio and use of absorbent filter media could be methods to further mitigate ammonia inhibition. As with cattle manure, co-digestion may also shorten SRT and enhance phase separation and thus methane yield and content. Therefore, research should be conducted considering other manures in LBR-AF systems.

#### 3.4. Macroalgae and aquatic weeds

The macroalgae Ulva and aquatic weeds Ipomoea carnea and water hyacinth have been investigated as feedstocks for LBR-AF systems. As discussed in Section 2.7, for a particular type of filter medium, successful phase separation (~80% methane produced in the AF) was achieved for macroalgae as it is readily biodegradable; however, high hydrogen sulfide concentrations (0.2-0.6%) were produced as the feedstock is sulfur rich [44]. Therefore, for macroalgae to be viable as a feedstock, research is needed to reduce hydrogen sulfide production and minimise the need for biogas purification. The use of a biochar filter could be one potential method to reduce hydrogen sulfide production from macroalgae in LBR-AF systems. This is discussed further in Section 5. In contrast to macroalgae, the digestion of water hyacinth in an LBR-AF system resulted in poor phase separation due to low rates of VFA production promoting methanogenesis in the LBR [93]. This indicates that slowly degrading weeds are not suited for mono-digestion in LBR-AF systems. Finally, for the digestion of Ipomoea carnea, singlephase LBRs (storing leachate internally or externally) with or without coupling to AFs were compared [45]. A three-fold increase in methane production was observed when coupling LBRs to AFs. However, details on phase separation were not reported. Overall, these studies demonstrate that there is potential for the use of macroalgae or aquatic weeds as feedstock in LBR-AF systems, but the research is limited compared with other feedstocks.

# 4. Leachate percolation in the LBR

To optimise LBR-AF performance there is a need to understand the influence of leachate percolation in the LBR on substrate degradation and VFA production, which were identified in Sections 2 and 3 as key factors influencing phase separation. This section draws knowledge from single-phase LBR studies that have investigated feedstock hydrodynamics, different strategies to enhance leachate percolation, and variation of leachate recirculation parameters.

#### 4.1. Feedstock hydrodynamics

To optimise substrate degradation and VFA production in the first phase of an LBR-AF system, there is a need to understand how leachate flows through the feedstock during anaerobic digestion. Although percolation through a solid porous material is generally well understood, feedstock hydrodynamics are more complex due to the dynamic properties of the degrading substrate. For example, as cattle manure degrades and compacts, decline in macro-porosity reduces permeability [94,95]. Consequently, after 30 days, leachate was observed to flow around the boundary rather than percolating through cattle manure, making recirculation unnecessary [96]. This indicates that leachate recirculation should be tailored based on the evolution of feedstock hydrodynamics [95]. Similar to cattle manure, for OFMSW [97] and chicken manure [98], permeability has been observed to decline with compaction. Furthermore, at large scales, the mass of feedstock heaps can result in compacted, impermeable zones within the feedstock [97,98]. It should be noted that feedstock characteristics and thus permeability can also vary with source; for instance, chicken manure permeability varies based on storage age, type of housing and bedding material [98]. These findings indicate that further research quantifying the hydrodynamics of different feedstocks is needed. Such knowledge should provide a basis to develop strategies to optimise leachate percolation and recirculation in an LBR. Studies have trialled different strategies, as will be discussed in Sections 4.2 and 4.3, but improved knowledge on feedstock hydrodynamics should assist with conducting systematic research to obtain more informative results. This would be further aided by the development of standard methods for quantifying feedstock hydrodynamics [99,100].

#### 4.2. Strategies to enhance leachate percolation

Various strategies have been trialled to enhance leachate percolation through feedstock in an LBR. These strategies include the addition of structural materials or co-substrates to feedstock, and the use of feedstock compartments. This section will discuss findings from studies that have considered these strategies.

The addition of structural materials to feedstock has been investigated as a strategy to enhance leachate percolation. For cattle manure digestion, the addition of wood powder (<1 mm) to feedstock at a volumetric ratio of 1:4, and wood chips (2-3 mm) at volumetric ratios of 1:2 and 1:1, have been compared [87]. Poor leachability (leachate produced as a percentage of influent volume) was observed for wood powder, presumably as small particle size resulted in little to no porosity enhancement. Conversely, the larger wood chips at both ratios improved leachability. For food waste digestion, the addition of five structural materials (1:10 volumetric ratio) has also been investigated: bottom ash, plastic full particles, plastic hollow spheres, saw dust and wood chips [101]. No clear relationship between particle size and leachability was observed. For example, bottom ash (best) outperformed similarly sized sawdust, and mid-sized wood chips (second best) outperformed the larger plastic full and hollow spheres. This indicates that factors other than particle size, for instance buffering capability [101], may influence leachability. In contrast to these studies, permeability has been directly quantified while investigating the influence of structural material addition to OFMSW [97] and chicken manure [98]. These studies used maize silage as a reference for good permeability as it is commonly used in full-scale dry digestion plants. Permeabilities comparable to maize silage were observed for the addition of 15% brushwood (by mass) to OFMSW [97], and 5% straw or 10% wood chips added to impermeable chicken manure [98]. Overall, these studies demonstrate that the addition of structural materials to feedstock can enhance leachate percolation; however, this practice significantly reduces organic loading in LBRs. This promotes the development of alternative strategies that do not compromise organic loading.

Co-digestion may be a strategy that improves percolation without compromising organic loading. For example, the addition of pistachio shells to cattle manure has been observed to increase VFA production by 193% compared to cattle manure mono-digestion [102]. Although the shells were considered inert in this study, pistachio shells are biodegradable [103] and therefore likely acted as both a structural material and co-substrate. In another cattle manure co-digestion study with grass, the use of certain filling methods has been observed to enhance percolation [104]. Layering of co-substrates improved percolation but mixing resulted in little to no percolation, as evidenced by substrate degradations of 72 and 50%, respectively. Therefore, depending on co-substrate characteristics, the filling method may impact the influence of co-digestion on leachate percolation. Overall, these studies demonstrate that co-digestion may be able to enhance permeability without compromising organic loading rate. However, further research is needed to better understand and optimise co-digestion as a strategy to enhance leachate percolation.

Another strategy that may be able to enhance percolation with minimal impact on organic loading is compartmentalisation; the separation of single feedstock heaps into smaller compartments stacked vertically in the LBR. The premise behind this approach is that compaction due to the mass of the feedstock heap is reduced through feedstock division between compartments. For food waste digestion, an LBR with three compartments has been investigated [105]. Compared with an LBR without compartments, increased substrate degradation and VFA production were observed due to reduced clogging. However, for digestion of an energy crop, poor leachate distribution has been observed in the lower of two compartments [106]. Therefore, although compartmentalisation shows some promise for enhancing leachate percolation, further investigation of how leachate is distributed between layers is required.

In summary, the addition of structural materials to feedstock has been demonstrated to enhance leachate percolation. However, structural materials compromise organic loading rate in the LBR. The use of suitable co-substrates rather than structural materials may provide similar percolation enhancement without compromising organic loading rate. However, for either of these strategies to be viable, they would likely require the use of waste materials that can be sourced locally and cheaply. Compartmentalisation is benefited in this regard as sourcing of materials is not required. Regardless of the method used, further research is required to develop these strategies and evaluate their use for different feedstocks. As these strategies improve leachate percolation and therefore enhance hydrolysis and VFA production, their application in LBR-AF systems may enable longer suppression of methanogenesis in the LBR, thereby enhancing phase separation. This highlights the importance of considering feedstock hydrodynamics and leachate percolation in further research investigating LBR-AF systems.

#### 4.3. Leachate recirculation parameters

To gain insight towards an optimal leachate recirculation strategy, various recirculation modes and parameters have been investigated for single-phase LBRs. Trickling and flood-and-drain leachate recirculation modes have been compared for different feedstocks. The degradation of horse manure [107] and swine bedding [108] were found to be insensitive to flow mode. However, flooding was recommended for horse manure as solid inoculum was not required [107], and trickling was recommended for swine bedding to minimise mobilised particles in leachate [108]. In contrast, trickling was observed to enhance the rate of hydrolysis for grass silage [109]. Therefore, feedstock properties may determine the sensitivity of substrate degradation to flow mode. Further research could identify feedstock characteristics that are favourable for different flow modes, and further consider the influence of feedstock hydrodynamics (discussed in Section 4.1).

The influence of various recirculation parameters has been considered for trickling leachate recirculation in LBRs. Intermittent recirculation is recommended over continuous recirculation [110]. Continuous recirculation requires higher energy usage and can result in the accumulation of inhibiting substances such as ammonia [111] and VFAs [106,110]. Similarly, other parameters such as recirculated volume, leachate to substrate ratio and the interval between recirculation events have been shown to influence inhibitory substance levels and thus methane yield in single-phase LBRs [112,113]. As LBR-AF systems have different tolerance levels to inhibitory substances (e.g., the AF mitigates VFA accumulation), there is a need to investigate how different recirculation strategies influence LBR-AF system performance. For an LBR-AF system, recirculation of AF effluent promotes leachate-based inoculation (refer to Section 2.2). Therefore, it is likely that continuous recirculation, or regular recirculation of high volumes, would promote faster leachate-based inoculation and thus be detrimental to phase separation. Further research should consider the influence of recirculation parameters on leachate-based inoculation and inhibitor levels, in addition to hydrolysis and VFA production, and the effect this has on LBR-AF phase separation.

# 5. Potential of a biochar filter for LBR-AF systems

A growing body of research is demonstrating the potential of porous, conductive, carbonaceous materials such as biochar and activated carbon to enhance anaerobic digestion. Biochar and activated carbon are produced from biomass via thermochemical conversion processes including gasification, hydrothermal carbonisation, pyrolysis and torrefaction [114]. However, compared with biochar, activated carbon is expensive as it is typically produced at higher temperatures and with further activation processes that enhance adsorption capacity and electrical conductivity [115]. Even without activation, biochars generally still have favourable properties that include high specific

surface area, porosity (pore sizes can range from micro- to macropores), pH and cation exchange capacity [116]. Therefore, as biochar is more economically viable, it is of particular interest for enhancing anaerobic digestion. This section will outline the proposed mechanisms that enable biochar to enhance anaerobic digestion, discuss the application of biochar and activated carbon as filter media in anaerobic digestion studies, and consider the potential for application of a biochar filter in LBR-AF systems.

Although the potential of biochar to improve anaerobic digestion has been demonstrated, the mechanisms by which biochars enhance anaerobic digestion are not well understood. Biochar has been reported to alleviate environmental stresses and enhance hydrolytic activity, acetate production and methanogenesis (reduced lag time and increased methane content and yield) [117]. Alleviating acid and ammonia stresses may be two mechanisms that improve methane production. Biochar properties can be favourable for retaining microorganisms and developing biofilms [5,118] that enhance methanogen resistance to ammonia [119]. Furthermore, biological activity may be enhanced by biochar complimenting feedstock to increase the bioavailability of trace elements [120,121]. Acid inhibition may also be relieved by biochar buffering capabilities [122]. Furthermore, the rate of acid conversion may be enhanced by direct interspecies electron transfer (DIET) between syntrophic bacteria and methanogens attached to the conductive surface of biochar; rather than indirect electron transfer (IET) via electron-accepting intermediates [115,123]. Promotion of the hydrogenotrophic methanogenesis pathway via DIET may also enhance methane content through the use of carbon dioxide for methane production [124]. However, the DIET mechanism has been criticised for overuse in explaining unforeseen results [125]. For example, it has been proposed that the acceleration of IET by redox-active functional groups on biochar surfaces may be a more dominant mechanism than DIET [126]. Although further research is required to better understand the mechanisms, the potential of biochar use for enhancing anaerobic digestion has been demonstrated.

Biochar as a filter medium has been investigated in low-solids anaerobic digestion studies treating high strength grease trap water [18, 118,127,128]. For instance, biochar derived from corn cobs excel at developing and retaining well-balanced microbial communities in AFs at laboratory [118] and demonstration scale [127]. Biochar derived from wood has also been investigated; either alone [128], or attached to chitosan biopolymer discs [18]. The antimicrobial properties of chitosan were hypothesised to maintain thin biofilms reducing resistance to mass transfer; however, corn cob biochar showed better potential for methane recovery [18]. The regular wood biochar was also effective as a filter medium while trialling a passive pH control strategy [128]. Despite limited understanding of the influence of biochar on anaerobic digestion, these studies demonstrate that biochar can be used effectively as a filter medium in AFs. Therefore, biochar has potential as a filter medium in an LBR-AF system.

Low-solids digestion studies have also investigated AFs packed with granular activated carbon (GAC) for treatment of wastewaters. For the digestion of olive mill wastewater, a GAC AF effectively depleted toxic phenols while operating stably under varying OLRs [129]. Stable longterm operation (723 days) has also been demonstrated; though the ability to remove phenols declined, purportedly with reducing adsorption capacity due to biofilm growth on activated carbon surfaces [130]. Furthermore, ceramic porous cubes and GAC have been compared as filter media at various OLRs and temperatures [131]. For all conditions considered, methanogenic activity and depletion of phenol and VFAs were high for GAC, but insignificant for ceramic cubes [131]. Similarly, for digestion of a chemical wastewater, five filter media have been compared: anthracite, GAC, black and red tezontle, and sand [132]. In comparison with the other filter media, GAC improved biogas quality (methane content of 80%-85% compared with 71%-76%) and enabled methanogenic activity to occur at a six times higher OLR. Additionally, start-up of a GAC filter was observed to be four times faster than the

second best performing red tezontle filter [133]. Overall, these studies indicate that compared with other filter media, activated carbon promotes rapid development of microorganism communities and enhances resistance to inhibiting and toxic substances, which in turn enhance biodegradation rates and methanogenic activity. Therefore, this further indicates that a carbonaceous material filter has the potential to enhance LBR-AF system performance.

The aforementioned studies treating wastewaters with toxic substances highlight the potential of a biochar filter to mitigate the influence of process inhibitors accumulating in leachate on LBR-AF performance. For example, the addition of biochar to anaerobic digesters reduce hydrogen sulfide production while maintaining [134,135] or increasing the production of methane [136]. Elevated sulfide levels can be toxic to microorganisms and promote sulfate-reducing bacteria that outcompete methanogens for essential intermediates used for methane production [64,136]. Although the mechanisms need further clarification, hydrogen sulfide may be directly adsorbed, or dissociate to bisulfide ions for further reaction to form sulfurous compounds on the surface of biochar [134,135]. Furthermore, DIET may improve co-existence of methanogens and sulfate-reducing bacteria to enhance hydrolysis and divert essential intermediates from hydrogen sulfide to methane production [136]. Regardless, this demonstrates the potential of biochar to mitigate the effects of sulfides in LBR-AF systems. Biochar has also been considered as an adsorbent material, typically in fixed columns rather than anaerobic digesters, for ammonium [135,137-139], heavy metals [140,141] and salts [142]. Therefore, research should consider the ability of biochar to mitigate the influence of different process inhibitors in LBR-AF systems. Complexities such as competition between different inhibitors for adsorption sites [137], as well as the influence of process conditions such as pH, will have to be considered.

Granular activated carbon filters have also been investigated to enhance anaerobic digestion in an LBR-UASB system. A micro-aerated LBR coupled to an UASB with two internally installed GAC filter packs (near the top and bottom) has been investigated for treatment of OFMSW [143]. The filter packs were installed during start-up of the isolated UASB to counter observed acid accumulation and process instability. The filters relieved acid inhibition to enable stable biogas production from the UASB. Then, once coupled to the LBR, high biogas production with methane content between 80 and 90% was observed. Although no direct comparison with a system without filters was provided, these results indicate that the GAC filters promoted stable operation and likely enhanced methane production and content. Further research should be conducted to elucidate the effects of the filters. However, the assertion that the filters enhanced methane production and content is supported by findings in UASB studies that have added carbonaceous materials to sludge. Adding biochar [115] and GAC [144,145] to sludge has been reported to enrich microbial populations with methanogens and syntrophic bacteria partners that participate in DIET; resulting in accelerated start-up, enhanced conversion of VFAs, and improved methane production and content. Therefore, as an LBR-UASB system operates similarly to an LBR-AF system, it provides the best indication that a carbonaceous filter should enhance LBR-AF performance.

The potential for a biochar filter to enhance LBR-AF system performance is evident based on aforementioned findings from anaerobic digestion studies investigating biochar or activated carbon. However, the structural stability of biochar over long-term use in anaerobic digestion systems requires clarification [125]. As physicochemical properties of biochar vary significantly based on production conditions (e.g., heating rate, temperature and residence time) and parent material [5,116, 146,147], the structural stability of biochars will vary. Investigation of stability will be important for assessing the feasibility of biochars as a filter medium; though stable use of GAC for over two years [130] suggests that some biochars may be structurally stable for long-term use. Provided biochar is structurally stable, the evolution of biofilm

consortia with biochar re-use and the influence this has on performance requires investigation. This need is evident as the addition of recycled biochar with developed microbial communities to feedstock has been observed to both outperform [5,8] and underperform [7] pristine biochar in terms of methane yield and lag time. Further questions such as the impact of inhibitor adsorption on microorganism growth, and vice versa as biofilms limit access to pores and surface area, will need to be considered to determine the lifespan of biochars in LBR-AF systems.

An important aspect of investigating biochar as a filter medium will be considering the influence of biochar properties on LBR-AF system performance. Although enhanced anaerobic digestion has been demonstrated for many biochar parent materials, others have been reported to have detrimental effects. For example, the addition of wood pellet biochar to poultry manure reduced lag time and improved methane yield, but sheep manure and wheat straw biochar had detrimental effects [5]. Similarly, for co-digestion of food waste and sewage sludge, adverse effects were observed for bamboo biochar addition. but biochars produced from miscanthus straw, rice husk and sewage sludge reduced lag time and improved VFA conversion and methane yield [123]. Different effects have also been observed using biochars produced at different temperatures for a particular parent material. For example, for the digestion of sewage sludge, significant variation in methane yield has been observed when comparing the addition of nine biochars produced from three parent materials, each at temperatures of 400, 500 and 600 °C [148]. Similarly, for simulated food waste digestion, the addition of biochar produced from pine sawdust at 900 °C compared with 650 °C has resulted in greater methane yields [149]. Therefore, further research is needed to identify suitable biochars and understand how biochar properties influence anaerobic digestion. Perhaps isolation of the biochar from feedstock in the LBR-AF system could somewhat simplify interpretation of experimental results and more clearly elucidate the influence of different properties. If so, this knowledge could benefit the application of biochar in all types of anaerobic digestion systems.

#### 6. Discussion

Review of studies considering LBR-AF systems has highlighted that various process parameters affect phase separation and thus process efficiency of the two-phase system. This section will discuss which parameters are critical and can be controlled to influence LBR-AF performance. Furthermore, challenges and future directions for research are discussed.

# 6.1. Critical process parameters

Review of different feedstocks considered in LBR-AF systems has indicated that feedstock characteristics are a key determinant of phase separation. Feedstocks that rapidly degrade and have ongoing VFA production promote regulation of LBR pH that enhances hydrolysis and suppresses methane production in the LBR; thereby enhancing phase separation. Consequently, increased organic loading of the AF increases the production of biogas with higher methane content, and thus the efficiency of the two-phase system. Although certain feedstocks are particularly well-suited for phase separation in LBR-AF systems, controllable parameters can also influence substrate degradation, pH regulation, and thus phase separation and process efficiency of the two-phase system. A summary of the critical parameters discussed is provided in Fig. 4.

Feedstock characteristics can be optimised using co-digestion. Co-digestion of rapidly- and slowly-degrading substrates can enable suitable LBR pH regulation to promote hydrolysis, maintain phase separation and increase production of higher quality biogas. Besides the typical benefits of co-digestion, such as improved C/N and increased microbial diversity, adding co-substrates can also enhance percolation

of leachate through feedstock to improve hydrolysis and VFA production. As co-digestion does not compromise organic loading, it is preferable compared with inert structural materials to improve feedstock hydrodynamics. For feedstocks such as manures and OFMSW, a balanced C/N may also help mitigate ammonia inhibition. Related to feedstock hydrodynamics, recirculation strategy also influences LBR-AF inhibitor levels and phase separation.

In single-phase LBRs, leachate recirculation parameters such as pumping rate and frequency influence substrate degradation and inhibitor levels. Similarly, the recirculation strategy used in LBR-AF systems will influence inhibitor levels, substrate degradation, phase separation, and thus process efficiency. Unlike single-phase systems. as the AF strips VFAs from leachate, effluent recirculation buffers the LBR. If there is insufficient acid production, LBRs can rapidly become methanogenic, making the two-phase system inefficient as pH becomes sub-optimal for hydrolysis and VFAs are consumed for production of lower quality biogas in the LBR (limiting AF organic loading). Therefore, understanding of suitable effluent recirculation rates and frequency will be important for optimising substrate degradation, regulating pH and maintaining phase separation in LBR-AF systems. In addition to recirculation between phases, more complex systems can separately circulate leachate in LBRs and effluent in AFs. This additional process control can enable switching between leachate and AF effluent for improved pH regulation and substrate degradation in the LBR. Control of effluent recirculation in the AF (i.e., HRT) will also influence phase separation. This additional process control, and potentially mixing of leachate and effluent, might enable pH control free of chemical additives. However, improved process control increases operational complexity, maintenance, and capital and operating expenses. The influence of recirculation strategy on inhibitor levels, biogas production, and quality of digestate and leachate will also need to be considered for feedstocks such as manures and OFMSW where inhibition is a concern.

The selection of filter media type in LBR-AF systems can influence phase separation and inhibitor levels in leachate. Filter media properties, such as porosity and surface roughness, effect microorganism retention and biofilm formation; influencing methanogen washout and LBR inoculation, as well as microorganism activity and resistance to inhibiting substances. The use of adsorbent materials such as biochar could also mitigate inhibitor accumulation in leachate in LBR-AF systems. There is likely an interplay between recirculation parameters, pH levels and filter media properties that needs to be explored to understand the influence on inhibitor levels and LBR-AF performance.

Inoculation strategy also influences LBR-AF performance. It is preferable to recycle digestate and leachate as inoculum. There are benefits to inoculating with acclimated and developed microbial communities, such as enhanced tolerance to inhibiting substances. However, this practice promotes rapid methane production in the LBR and thus poor phase separation and process efficiency for LBR-AF systems. Improved pH regulation through co-digestion or recirculation parameters, in conjunction with consideration of feedstock-to-digestate ratios and the volume of recycled leachate, may be necessary to optimise phase separation and production of high-quality biogas in LBR-AF systems.

The temperature regime used for LBR-AF systems can also have implications on substrate degradation, phase separation and inhibitor levels. Coupling a thermophilic LBR with a mesophilic AF has been observed to improve process efficiency by reducing solid retention time and enhancing phase separation. The decline in temperature between the LBR and AF may inhibit methanogens transferred between reactors; though this requires further clarification. For feedstocks such as manure and OFMSW, elevated ammonia production with the use of higher temperatures should be considered. If higher temperatures are not feasible, manipulation of other critical parameters may enable successful phase separation at lower temperature regimes.

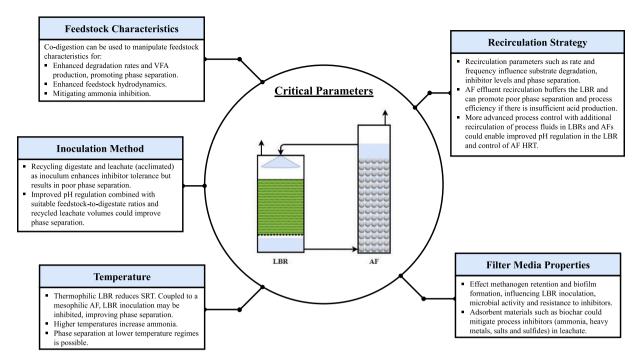


Fig. 4. Summary of critical parameters influencing phase separation and process efficiency for an LBR-AF system.

# 6.2. Challenges and future direction

Phase separation in an LBR-AF system can improve process efficiency by enhancing hydrolysis and acid production in the LBR, which promotes production of higher quality biogas in the AF. However, successful phase separation is often not achieved. Critical parameters that influence phase separation and process efficiency were highlighted in Section 6.1. Despite identifying critical parameters, many gaps in knowledge regarding the influence of process parameters on LBR-AF performance were identified that should be investigated in further optimisation studies. Potential research questions to address key gaps in knowledge have been provided in Table 6. Another factor that should be considered in future research is AF scale. Process instability due to excessive OLR in AFs coupled to LBRs was not reported. This indicates scope for optimising scale to use more compact AFs and reduce capital expenses. Implications of scale on performance such as the capacity and thus life span of adsorbent filter media should be considered. It should also be noted that there were no studies identified that directly compared LBR-AF systems with single-phase LBRs. Comparison of LBR-AF performance with single-phase LBRs is required to determine the economic viability of the two-phase system, considering the additional capital and operating expenses. However, further research and development of LBR-AF systems is suggested to gain better insight into optimal performance before economic evaluation.

This review outlines multiple research gaps that could be pursued to optimise biogas production from LBR-AF systems; however, digestate quality has been largely neglected as most literature focusses on enhancing biogas production. Although digestate and leachate quality is often overlooked, it should be considered important as treatment may be required to dispose of digestate safely as fertiliser (value-adding) or waste (an expense) [1,2]. For example, depending on feedstock characteristics, digestates may have high levels of unwanted compounds such as heavy metals or salinity that restrict use as fertiliser without treatment [1,30]. Therefore, if co-digestion is considered in LBR-AF systems, the influence of co-substrate selection on digestate quality should be considered in addition to the effect on biogas production. This example highlights that it is important for future research to consider trade-offs between biogas production and digestate quality when optimising LBR-AF performance and assessing economic viability.

Overall, review of the current literature has provided insight into the influence of process parameters on LBR-AF phase separation and process efficiency. The outlined benefits of a two-phase system and research gaps provide motivation for further research considering LBR-AF systems. Furthermore, review of literature investigating biochar and activated carbon as filter media in AFs, and as additives for LBR-UASB systems, has indicated the potential biochar has for use as a filter medium in LBR-AF systems. A biochar filter may promote rapid development and retention of enriched microbial communities; thereby accelerating AF start-up, improving resistance to inhibiting substances, and enhancing methane production and content. Furthermore, biochar as a filter medium may reduce process inhibitors in leachate. This provides clear motivation for investigation of biochar filters in LBR-AF systems. As biochar properties vary significantly with production conditions and parent material, and not all biochar types enhance anaerobic digestion, suitable biochars should be identified through investigating the influence of biochar properties on LBR-AF system performance. This path of investigation could simultaneously enhance understanding of the influence of filter media properties on phase separation and LBR-AF performance. For biochars identified as suitable, it would also be important to understand the evolution of biofilm consortia with extended filter re-use, as well as capacity to adsorb inhibitors, and the influence these have on LBR-AF performance. Assuming biochar filters are found to enhance LBR-AF performance, knowledge on suitable biochars and filter lifespan would be important factors in evaluating the economic viability of biochar filters in LBR-AF systems.

#### 7. Conclusion

The benefits of LBR-AF systems compared to single-phase LBRs include improved stability and process efficiency (improved hydrolysis and higher methane yield and content), provided phase separation is successful. Feedstock characteristics are a key determinant of phase separation. Feedstocks most suited for phase separation in LBR-AF systems degrade rapidly with ongoing VFA production that regulates LBR pH to promote hydrolysis, suppress methane production in the LBR, and maximise AF organic loading. However, process parameters can also be manipulated to influence phase separation. Critical parameters that can be controlled include co-digestion, recirculation parameters, filter

Table 6
Potential research questions addressing identified knowledge gaps.

Parameter	Potential research questions
Feedstock Characteristics	<ul> <li>For different feedstocks, how can co-digestion be used to optimise substrate degradation rates for improved phase separation and process efficiency? What is the influence on process inhibitor levels?</li> <li>How does co-digestion influence feedstock hydrodynamics in LBR-AF systems?</li> <li>Can LBR-AF systems enhance digestion of livestock manures (other than cattle) where inhibition is more of a concern? Do inhibitor mitigation</li> </ul>
	strategies help?
Filter Media	<ul> <li>How do filter media types and properties influence phase separation and process efficiency?</li> <li>Can adsorbent filter media mitigate inhibitor levels in leachate? How does this effect performance?</li> <li>Is biofilm growth affected by inhibitor adsorption, and vice versa? How long would filter media remain effective?</li> <li>How does the application of a biochar filter in an LBR-AF system influence phase separation, process inhibitors and system performance?</li> <li>Is there an interplay between recirculation parameters, pH and filter media properties on inhibitor mitigation?</li> </ul>
Recirculation Strategy	<ul> <li>What is the effect of recirculation parameters (e.g., pump rate and frequency, AF HRT) on phase separation and inhibitor levels? How does this vary for different feedstocks?</li> <li>What is the tolerance level of LBR-AF systems to process inhibitors compared to single-phase LBRs?</li> <li>Can more advanced process control (leachate recirculation in the LBR, effluent recirculation in the AF and potentially mixing of fluids) enable enhanced pH control and improved phase separation and process efficiency? Does the improvement outweigh additional costs and complexity?</li> </ul>
Temperature	<ul> <li>What is the mechanism behind the temperature differential between a thermophilic LBR and mesophilic AF influencing phase separation in LBR-AF systems? Does this apply for different feedstock types?</li> <li>How does temperature influence ammonia levels for feedstocks such as manure and OFMSW in LBR-AF systems? What are the trade-offs between increased substrate degradation, inhibitor levels and LBR-AF performance?</li> <li>How does application of lower temperature regimes (mesophilic or psychrophilic, controlled or uncontrolled) influence the ability of other process parameters to effect phase separation and process efficiency?</li> </ul>
Inoculation Strategy	<ul> <li>How can inoculation strategy be optimised to reduce negative impacts on phase separation and process efficiency?</li> <li>Can improved pH control (e.g., co-digestion and/or recirculation strategies), combined with selective feedstock-to-digestate ratios and volume of recycled leachate, enhance phase separation?</li> </ul>
Pressure	<ul> <li>Can improved pH control prevent methane yield decline with increasing methane content in pressurised AFs?</li> <li>If AF effluent is recirculated to an unpressurised LBR (like a flash tank), what are the effects on LBR-AF phase separation and process efficiency</li> </ul>

media properties, inoculation strategy and temperature. Depending on feedstock type, these parameters can also have implications for process inhibitor levels in LBR-AF systems. Identified research gaps relating to these critical parameters provide paths for future research considering optimisation of LBR-AF phase separation and process efficiency. Future optimisation studies should consider trade-offs between biogas production and digestate quality, as the ability to use or sell fertiliser can have significant economic implications. Furthermore, comparison of optimised LBR-AF performance with single-phase LBRs is required to determine the economic viability of the two-phase system, considering the additional capital and operating expenses. Finally, biochar application in other anaerobic digestion systems demonstrates potential benefits for use as a filter medium, such as rapid development of enriched microbial communities, enhanced methane yield and content, and mitigation of process inhibitors. This provides justification for further research considering high-solids anaerobic digestion in an LBR coupled to a biochar-packed AF.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

No data was used for the research described in the article.

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