

Review

Anaerobic Digestion of Blood from Slaughtered Livestock: A Review

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Abstract: Blood from livestock slaughtering imposes a high organic pollution load and risks. If it is discharged untreated to sewer systems, it increases the organic pollution load on wastewater treatment plants by 35–50%. This paper reviews blood anaerobic digestion. It analyzes the quantities, composition, methane potential reported, microbiology, biochemical pathways of blood protein degradation, environmental and health issues, and strategies suggested to manage them during livestock blood anaerobic digestion. Although challenging, anaerobic digestion of blood as a mono-substrate is possible if the culture-reactor system is controlled based on a complete characterization and understanding of the microbial community and its metabolic activities. Co-digestion of blood and other feedstock proceeds well if the mixtures are well designed. Generally, the specific methane yield from digesting blood alone ranges between zero and 0.45 m³ kg⁻¹ protein, whereas for co-digesting blood and other substrates, the yield varies between 0.1 and 0.7 m³ kg⁻¹ volatile solids. More research is required for microbiology and kinetics, the role of adsorbents, reactor configuration, and culture adaptation during anaerobic digestion of blood to better control the process.

Keywords: blood; livestock blood; slaughterhouse waste; protein; anaerobic digestion; biogas



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1. Introduction

The livestock animal-slaughtering process generates large quantities of blood as a byproduct of high nitrogen content and high chemical oxygen demand (COD). Blood's total nitrogen is around 30 g L⁻¹, and the COD population equivalent (PE) of blood from a cattle carcass is 50 g L⁻¹ [1]. It has been found that direct discharge of untreated blood in the sewer system increases the organic pollution load on the wastewater by 35–50% [2,3]. Therefore, blood's high organic pollution load poses risks to the environment if discharged or disposed of without proper treatment [4,5]. Among the risks, discharging untreated blood to water streams depletes the dissolved oxygen, enriches the nutrients content, induces septic conditions, and spreads microbial and viral waterborne diseases [6,7]. Therefore, blood collection and treatment are the best approaches to manage, control, and decrease the pollution caused by slaughterhouses wastewater [8].

The United Nations' Food and Agriculture Organization (FAO) estimated that more than 49.2, 70.3, and 74.2 million animals were slaughtered in 2003, 2014, and 2016, respectively (Table 1). This number is growing steadily according to the Global Slaughter Index [9]. All animals slaughtered bleed to various extents during the slaughtering process, depending on the type of animal, its size, slaughtering method, and the duration of blood collection.

Table 1. The number of animals slaughtered worldwide in 2003 and 2016 [9].

Animal Type	2003 (10 ⁶ Head)	Percentage	2016 (10 ⁶ Head)	Percentage
Chickens	45,895	93.1	65,847	88.8
Ducks	2262	4.3	3065	4.1
Pigs	1244	2.4	1480	2.0
Rabbits	857	1.6	981	1.3
Turkeys	691	1.3	673	0.9
Geese	533	1.0	659	0.9
Sheep	515	1.0	551	0.7
Goats	345	0.7	460	0.6
Cows and calves	292	0.6	302	0.4
Other rodents	65	0.1	70.4	0.1
Pigeons and other birds	63	0.1	55.3	0.1
Buffalo	23	0.04	26.2	0.4
Horses	4	0.01	4.78	0.01
Donkeys and mules	3	0.006	3.05	0.004
Camels	2	0.004	2.45	0.003
Total	49,292		74,180	

In a typical US poultry processing plant, around 200,000 birds are slaughtered daily, generating about 5200 m³ of wastewater and 13.6 m³ of blood, assuming that blood recovery from the slaughtered animals is around 40% [10,11]. The US poultry industry slaughtered around 8.6×10^9 broilers (2.7 kg average weight) in 2013 [2]; this is equivalent to 1.76×10^9 kg of blood, assuming that blood forms 7 to >11% of the broiler live weight for 3.0 and 1.0 kg live-weight birds, respectively [11].

Processing of blood requires specialized technical facilities. For example, there are only 11 blood-processing plants in the EU [12]. One blood-processing plant exists in Belgium, Denmark, Spain, France, the Netherlands, Italy, and Sweden, whereas Germany and the UK have two plants each [12]. Together, these 11 blood-processing plants processed about 300×10^9 metric tons of blood [12]. In Spain, for example, the lack of such facilities in or within a reasonable distance of slaughterhouses rendered treating liquid blood relatively difficult. Therefore, coagulated blood is usually shipped to be incinerated after heat dewatering [13].

On the other hand, blood is an organic matter composed of mainly protein. It is a potential feedstock for biogas production after some pretreatment or proper management and control of the fermentation process. The highly degradable protein content (94–96%) of blood plus its lipid/fat content (3% of the volatile solids (VS)) contribute to its high potential methane yield (500 L kg⁻¹ VS) [14,15]. Afazeli et al. [16] estimated that out of the 54×10^6 m³ annual biogas yield potential from slaughterhouse waste in Iran, blood contributes about 31%. Based on an experimental work, Wang [2] estimated that anaerobic digestion (AD) (at 35 °C and an HRT of 7 days) of 1:3 (v/v) of poultry blood and poultry-processing wastewater produced from slaughtering 200,000 birds/day would generate 146.2 m³ CH₄ d⁻¹. The net energy recovery would be 1.5 GJ d⁻¹, while nitrogen (N), phosphorus (P), and potassium (K) recovery would be 251.5, 3.0, and 3.7 kg d⁻¹, respectively, in a relatively small volume bioreactor (530 m³).

Blood is organic biomass that can be fed to an anaerobic digester to generate combustible biogas. To the authors' best knowledge, no previous article has comprehensively reviewed livestock blood AD, focusing on its conversion to biogas. This paper aims to review the accessible literature on AD of slaughtered livestock blood and its conversion to biogas to better understand this topic's state of the art. It covers various theoretical and practical aspects of slaughterhouse blood quantities, characteristics, composition, theoretical and experimental methane yields in batch and continuous reactors studies, concerns faced during blood AD and strategies considered to control and manage these issues, biochemical pathways, microbiology of blood anaerobic degradation in digesters, and the

kinetics of blood AD. The paper identifies the knowledge gaps and suggests directions for future research. Figure 1 provides a schematic overview of the article's content. The journal *energies* is a platform for research published on energy-related topics, and the current review fits herein. The review's topic is of interest for professionals working in environmental, chemical, and process engineering; microbiology and bioenergy technology; and the academic, research, and governmental sectors.

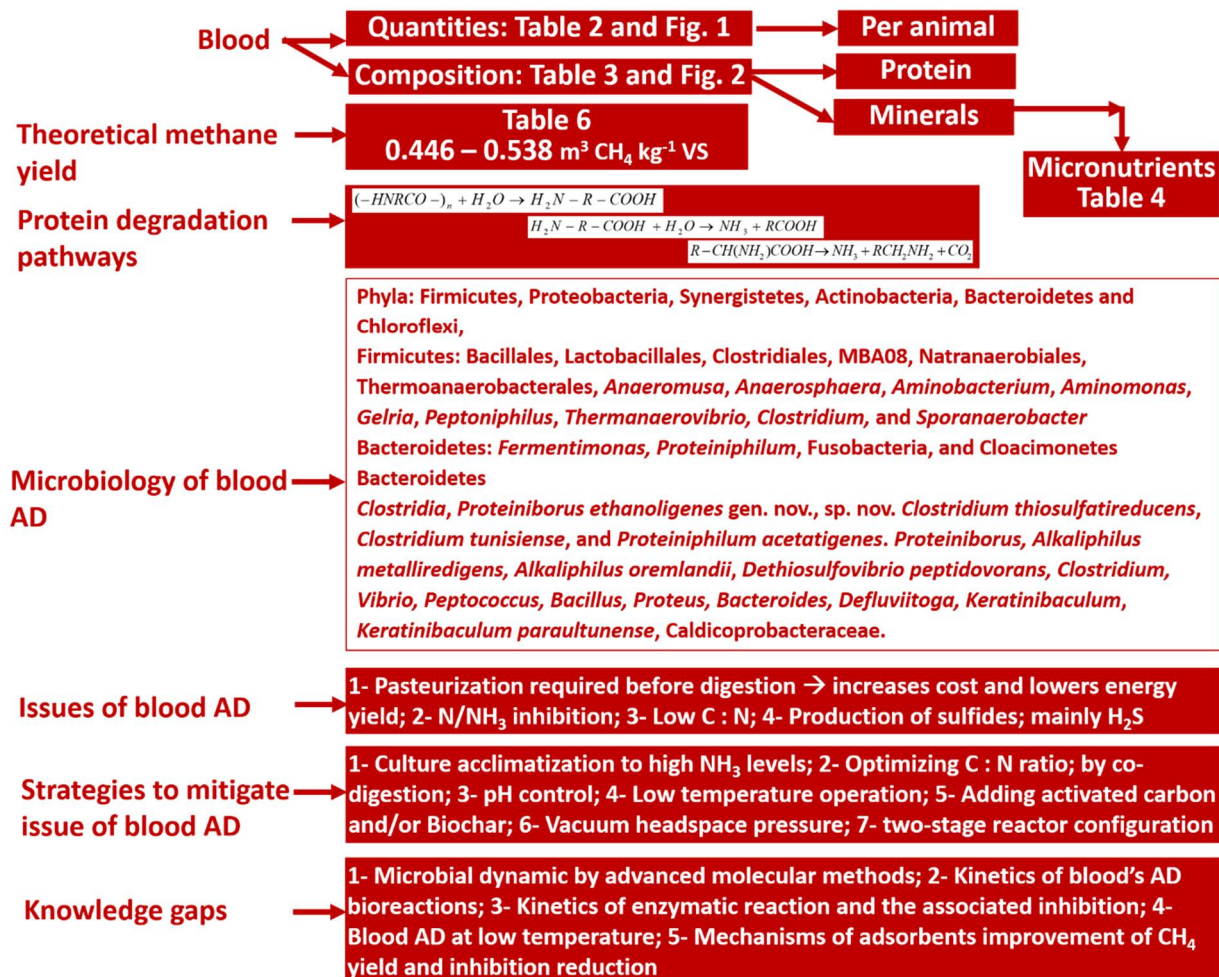


Figure 1. Schematic overview of the review content.

2. Quantities of Blood from Livestock Slaughtering

In 2010, the number (in millions) of livestock animals slaughtered and processed for meat production worldwide was around 304 (cattle), 959 (sheep and goats), and 1374 (pigs) [2,9]. Bah et al. [4] estimated the volume (liter) of blood from these animals to be around 4.56×10^9 , assuming that the volume (liter) of blood recovered per head slaughtered is 15 (cattle) and 2–3 (pig) [17]. The European Community report [12] indicated that 10–20 L (cattle) and 2–4 L (pig) of blood is collected from each animal slaughtered [12].

In the US, about 162 processing plants slaughter 200,000 broilers per day [2,11]. Only 75% of the bird's live weight, when slaughtered, ends up as edible meat while the rest (25%) is organic offal inedible byproducts such as feather, blood, and intestine residues [11,14,18]. This applies to almost all types of the animals slaughtered.

Upon slaughtering livestock animals, the typical bleeding time is: 6 min (cattle), 4–5 min (sheep), 3–4 min (calves), 6 min (pigs), and 2–3 min (poultry; broiler) [11,15,19]. Accordingly, only 0.5 to 0.9×10^9 kg of blood could be collected annually in the US [2]. Slaughtering 181,770 cattle and 237,300 goats generated 2462 and 2518 tons of blood waste,

respectively [20]. Around 125 livestock animals (55 cattle, 50 sheep, and 20 goats) that were slaughtered daily at Tamale abattoir (in Ghana) produced 0.7 metric ton of blood per day [21].

The slaughtering process generates inedible organic offal byproducts. Figure 2 gives the quantities of blood generated from slaughtering cows, pigs, sheep, and poultry as a percentage of the live weight (Lv Wt) or live body weight (Lv Bw), mass per head, and volume in liter. Blood forms about 2.4–8.0% of the animal's live weight, depending on the animal type (Figure 2) [22,23], and about 6% to 7% of the lean meat content of the carcass [23]. Blood forms 2.5% of the live weight in cows and 0.6% in sheep and goats [24,25]. Afazeli et al. [16] reported that the blood forms 7.0%, 3.0%, and 3.0% of the whole bodyweight of heavyweight (250 kg), lightweight (40 kg), and poultry (1.5 kg) slaughtered animals, respectively. Approximately 10–22 kg of blood is collected per head of cattle slaughtered [26,27], and approximately 40 g per broiler (2% of the live weight) [28]. However, Jayathilakan et al. [22] reported that blood forms about 3.2–3.7% of the live weight of poultry slaughtered.

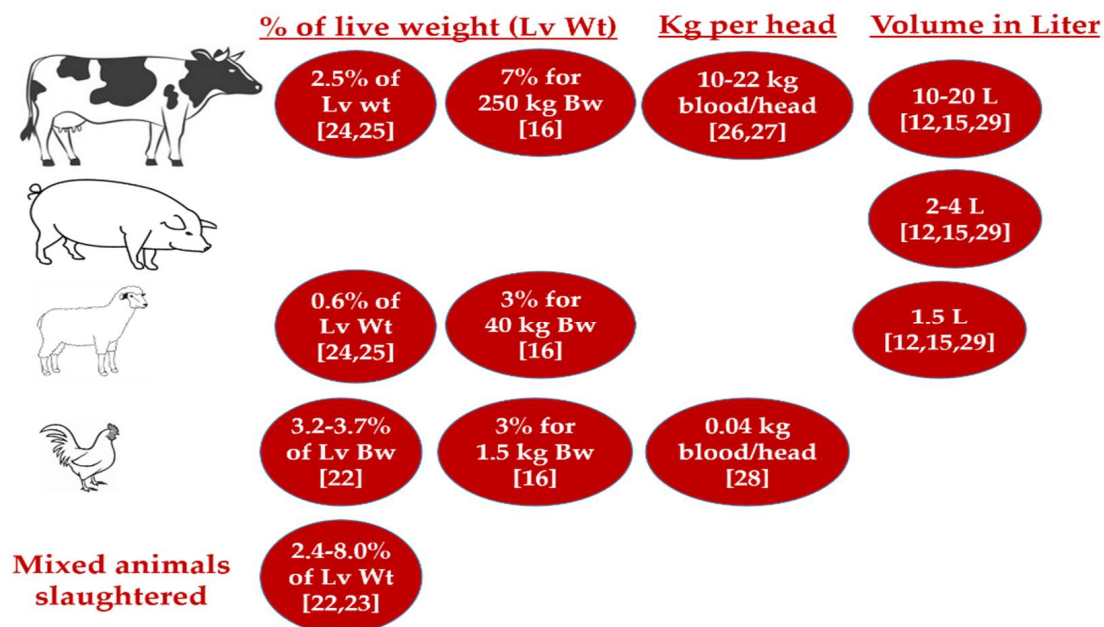


Figure 2. Quantities of blood generated from animal slaughtering. Lv: live; Bw: body weight. The numbers in the brackets are citations.

The mass of blood is a function of the species of an animal and its live weight (Table 2). In Iran, 17% of the blood produced at slaughterhouses is from heavy livestock, 14% is from poultry, and 5% is from light livestock [16]. Generally, slaughterhouses recover only a small fraction of the generated blood. On average, 3.0–4.0%, 3.0–4.0%, and 3.5–4.0% of blood are recovered from pigs, cattle, and lambs, respectively [22]. The typical volume (in liter) of blood collected after slaughtering an animal is 2–4 (pig), 1.5 (sheep), and 10–20 (cattle), and around 10% of the body weight of the chicken [12,15,29]. For example, a typical 450 kg live weight commercial steer yields about 16 kg of blood [29]. Singleton [30] measured 13.3 L per head of cattle slaughtered.

Around 50% of the bleeding blood is collected after slaughtering, while the rest is retained in the carcass [15]. When slaughtering a broiler, about 30–50% of the animal blood drains and could be collected, and 70–50% remains in the carcass and is partially washed out during the cutting process [11,19]. Using vacuum pressure to collect the blood could increase the volumes of blood collected, since vacuum pressure shortens the time of blood collection while recovering more blood from the carcass.

Blood is mainly protein with a biochemical oxygen demand (BOD) of 140–200 g L⁻¹ [31,32] and COD as high as 400 g L⁻¹ [29]. For example, the COD of raw bovine blood is up to 375 g L⁻¹ [8]. Therefore, blood or its diluted wash water from slaughterhouses is a wastewater stream of high organic pollution load [4,5]. The efficiencies of blood separation, collection, and recovery facilities installed at slaughterhouses determine the BOD and COD of their wastewaters [22]. Wu and Mitta [33] found that 62% of the slaughterhouses in Ontario collect blood separately; 87% of them send it to rendering plants, while 13% compost it on site. Separating the blood from the slaughterhouse wastewater decreased its BOD₅/COD ratio from 0.53 to 0.46 (beef), 0.42 to 0.31 (pork), 0.67 to 0.47 (sheep), 0.38 to 0.33 (mixed), and 0.46 to 0.21 (poultry); consequently, it decreased the odor of the wastewater [33].

Table 2. Quantity of blood produced from animal slaughtering.

Animal	Live-Weight (kg)	Wet Blood (kg)	Reference
Beef cattle	454	14.5	[34]
Beef cattle	453	14.7	[35]
Beef cattle	450	16	[36]
Cattle	270	19	[37]
Cow	Not reported	13.6	[38]
Cow	250	12.7	[21]
Heavy livestock	250	21	[16]
Light livestock	40	1.2	[16]
Pig	28	3	[37]
Pig	Not reported	3	[22]
Sheep and Goat	22	0.72	[21]
Sheep	20	0.48	[22]
Poultry	1.5	0.045	[16]
Chicken	2.28	0.024	[39]

Blood is usually used as a raw material to make blood meal, fibrin or blood serum, fertilizer, and/or animal feed. Blood is rich in carbon and nutrients, and can be used as feedstock for AD to recover the energy impeded in blood protein by converting it into biogas, heat, and electricity.

3. Composition of Blood Waste

Blood is a red fluid that contains water, cells, enzymes, proteins, and other organic and inorganic components (Figure 3). Once outside the blood vessels, blood coagulates in 1–2 min [40,41]. Upon storage, blood stratifies after a long period due to the settling of the heavier blood components. Therefore, mixing is inevitable during treatment processes such as AD.

Blood comprises the cellular matter (red blood cells, white blood cells, and platelets forming 30–40% of the blood wet-mass) dispersed in a liquid called plasma (about 60–63%) [4,22]. The cellular matter is 90% protein composed of mostly hemoglobin. Plasma, a yellow liquid, is 6–8% proteins such as albumin (3.5%), globulins, and fibrinogen (4.0%), plus more than 100 smaller proteins [4,22]. The density of blood is usually about 1.0 kg L⁻¹; however, the density of bovine blood can be as high as 1.5 kg L⁻¹ [38]. In terms of the blood's cellular matter content (% by weight of blood): pigs (43.5%) > cattle (32.5%) > sheep (23%); whereas in terms of plasma content (% by weight of blood): sheep (72%) > cattle (67.5%) > pigs (56.5%) [4,42]. Hemoglobin and albumin form the largest proportion of proteins in the blood. Hemoglobin forms 14.2%, 10.3%, and 9.3% of the proteins in the blood of pigs, cattle, and sheep, respectively, and albumin forms on average 3.8% [4,42].

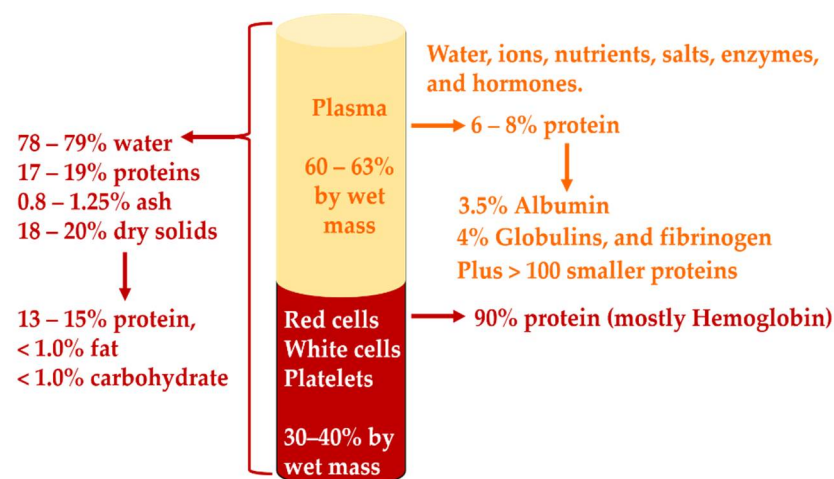


Figure 3. Components of slaughtered animals' blood. The figure is based on data from [4,14,15,18,22,28,43–54].

3.1. Blood Protein Content

Table 3 summarizes the reported physio-chemical characteristics of livestock blood. Blood composition varies slightly depending on the type of animal (Table 3). Blood has total solids (TS) of 10.8–23%, and volatile solids (VS) form 91–96.6% of TS. Generally, blood has a high protein content (as high as 965 g kg⁻¹ dry organic matter or 94.4% of VS); consequently, its COD is relatively high [12].

Blood from healthy animals is sterile and contains an excellent quantity of amino acids [15,22]. According to the data compiled in Table 3 for blood characteristics from poultry, swine, sheep, cattle, and mixed animals, blood is 17–19% proteins, 18–20% dry solids, 78–79% moisture content, and 0.8–1.25 ash. Blood dry solids are 13–15% protein, <1.0% fat, and <1.0% carbohydrate with a pH of 7.4 (Table 3). Therefore, blood has a high nitrogen (N) content (>15 kg m⁻³); for comparison, undiluted dairy cow slurry contains 4 kg N m⁻³ [43]. Blood contains around 3.5 g NH₃-N L⁻¹; this is around 270 times that of domestic wastewater (4 to 13 mg L⁻¹) [44–47]. Thus, blood has a very high population equivalent in terms of pollution due to nitrogen. Slaughterhouse wastewater containing blood has total nitrogen 2.3 to 4.4 times that of typical domestic wastewater [45–47]. The high concentrations of proteins and associated nitrogen discussed above increase the pollution load of slaughterhouse wastewater, mainly due to the large quantity of blood in the wash water. For example, meat rendering and processing require water (m³ metric tonne⁻¹ of carcass) at the rate of 1.5–10 (pigs), 2.5–40 (cattle), and 6–30 (poultry) [38,48]. After processing, this water becomes polluted with blood and needs treatment before disposal.

Table 3. Typical characteristics of livestock blood.

	[15]	[28]	[54]	[18]	[50,52]	[51]	[14]	[55]	[56]	[57]	[58]	[59]	[51]	[60]	[61]	[49]
Animal	Poultry	Poultry	Poultry	Poultry	Sheep	Swine	Pig	Pig	Pig	Pig	Pig	Cattle	Cow	Cow	Mixed	Mixed
TS (%)	18–20	22	10.8	132.4 g kg ⁻¹	19.7	22.2	17.9	18–22	19.7	18	23.32	13.5	23	19.8	20.90	
VS (%)			9.3	125.9 g kg ⁻¹	95.6		16.8	17–21	18.4	17				75	20.19	
VS/TS		0.91								0.944	0.958		0.9636		0.966	
C/N												3.2			3.4	
C (%)			34.2	49.1% w/w	42.1% oTS					48.3	168.7 g kg ⁻¹	49.7			51.27	
H (%)				7.2% w/w	7.3% oTS					9.0					6.95	
O (%)				23.8% w/w	27.1% oTS					24.8					22.68	
N (%)				14.4% w/w		8.32% oTS	25.4			14.9%	52.66 g kg ⁻¹	15.8	15% oTS	2.9	15.04	
S (%)				0.6	1% oTS					0.4					0.66	
Kjeldahl N		7.6% oTS	12% db	136 g kg ⁻¹	147 g kg ⁻¹		27.1 g kg ⁻¹	28–32	31.7 g kg ⁻¹							16.5 g N L ⁻¹
TP				118 mg kg ⁻¹	0.84 g kg ⁻¹	0.49 g kg ⁻¹						0.61	0.87 g kg ⁻¹			0.183 g L ⁻¹
Dissolved NH3			16.6 g l ⁻¹				1.7 g N kg ⁻¹		0.1 g N kg ⁻¹							3.5 g L ⁻¹
Ash (%)	0.8–1.2						24.5					93.2			3.40	
Protein	18–19%	48% oTS	7.5% db	89.69% w/w	965 g kg ⁻¹		94.4% oVS		197.3 g kg ⁻¹	93%	94.4% oDVS					81.25 g L ⁻¹
pH	7.4				7.23	7.25						6.8	7.4	7.4		
Fat/lipids	<1.0%	2% oTS	0.3% db	3.05% w/w	<10 g kg ⁻¹		0.3% oVS			1.1	0.3% oDVS					
CHO	<1%				7.2 g kg ⁻¹		5.3% oVS									
TOC					42.0% oTS						5.3% oDVS					

Note: TS: total solids; VS: volatile solids; C/N: carbon:nitrogen ratio; C: carbon; H: hydrogen; N: nitrogen; S: sulfur; TP: total phosphorus; O: oxygen; CHO: carbohydrates; TOC: total organic carbon; oTS: of TS; oDVS: of dry VS; oVS: of VS.

3.2. Blood Mineral Content

Table 4 summarizes data reported in the literature on the mineral content of livestock blood: phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), cobalt (Co), sulfur (S), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). Collectively, they constitute about 4.8% (by dry weight) of animal blood [2,18,49–53].

Table 4. Micronutrient contents in slaughtered animal blood.

Element (ppm ¹)	Broiler ^a	Sheep ^b	Sheep ^c	Cow ^d	Swine ^e	Pig ^f	Mixed ^{g2}
P	118	164					183
K	92.7	731		667.9	2380	118	798
Ca	44.9	NR		130	90	1221	55
Mg	5.4	NR				224,800	27
Na	148.3	NR		2763	1650	94	818
Fe	48	NR				368	164
Co	0.01	NR				0.1	<0.02
S	NR	NR	1% TS			4000	300
Cd	NR	<0.20					0.05
Cr	NR	<0.40					0.3
Cu	0.1	1.32				14.6	0.7
Ni	1	<1.0				1.6	1.3
Pb	NR	<2.0					
Zn	1.7	3.2				13	

Note: ^a—Yoon et al. [18]; ^b—Zhang and Banks [50]; ^c—Banks and Zhang [52]; ^d and ^e—Alvarez et al. [51]; ^f—Kim et al. [57]; ^g—Hansen and West [49]. ¹—ppm on a weight basis; ²—total chloride = 1700 mg L⁻¹; Ni < 0.02 mg L⁻¹; Pb < 0.6 mg L⁻¹; Mn = 0.05 mg L⁻¹.

Along with the protein content, the mineral content makes blood a very nutritive substrate for anaerobic mixed culture microorganisms mediating the bioreactions of AD.

4. Theoretical Methane Potential of Blood

Protein is a biodegradable matter with a typical chemical formula of C_cH_hO_oN_nS_s. It has a relatively high methane potential compared to carbohydrates and fats. Using the typical chemical formulas for carbohydrates (CH₂O), fats (C₈H₁₆O), and proteins (C₁₆H₂₄O₅N₄) [62], the half-reactions (Table 5) show that protein produces the highest specific molar yield of methane [62]. The stoichiometric specific methane yield is 8.25 mol CH₄ mol⁻¹ protein compared to 5.75 mol CH₄ mol⁻¹ for fats and 0.5 mol CH₄ mol⁻¹ for carbohydrates.

Table 5. Half-reactions for protein, fat, and carbohydrate conversion to methane.

Substrate	Half-Reaction	Equation
Proteins	$\frac{1}{66}C_{16}H_{24}O_5N_4 + \frac{27}{66}H_2O \rightarrow \frac{8}{33}CO_2 + \frac{2}{33}NH_4^+ + \frac{31}{33}H^+ + e^-$	(1)
	$\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	(2)
Fats	$\frac{1}{46}C_8H_{16}O + \frac{15}{46}H_2O \rightarrow \frac{4}{23}CO_2 + H^+ + e^-$	(3)
	$\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	(4)
Carbohydrates	$\frac{1}{4}CH_2O + \frac{1}{4}H_2O \rightarrow \frac{1}{4}CO_2 + H^+ + e^-$	(5)
	$\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	(6)

Note: The chemical formula and half-reaction derivation are based on Rittmann and McCarty [62].

Kim et al. [57] reported that the chemical formula for pig blood is C_{3.78}H_{8.39}O_{1.46}N₁S_{0.01} based on an elemental analysis. Similarly, Banks and Zhang [52] and Yoon et al. [18] used elemental analyses and derived chemical formulae for sheep and poultry blood, respectively (Table 6). Hidalgo and Martín-Marroquín [61] reported results of elemental analysis for pasteurized mixed bloods obtained from a slaughterhouse. Accordingly, the theoretical

maximum stoichiometric methane yield potential of the blood protein at standard conditions (STP; pressure: 1 atm and temperature: 0 °C) can be estimated using Buswell and Muller's formula (Equation (7)).

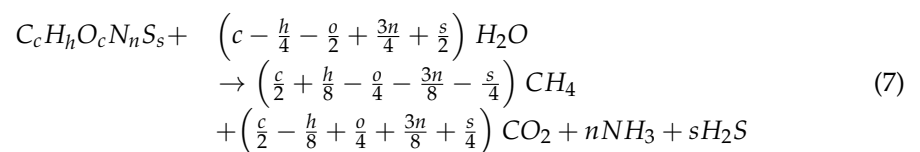
Table 6. Theoretical stoichiometric methane potential of livestock blood.

Type of Blood	Chemical Formula	B _{theoretical} ^a	B _u ^b	Biodegradability ^d (%)	Reference
Pig	C _{3.78} H _{8.39} O _{1.46} N ₁ S _{0.01}	0.538	0.443	82.3	[57]
Poultry	C _{3.98} H _{6.93} O _{1.45} N ₁ S _{0.018}	0.512	0.250	48.8	[18]
Sheep	C _{3.34} H _{6.88} O _{1.61} N _{0.03}	0.446		93.5 ^c	[52]
Mixed	C _{3.98} H _{6.4} O _{1.32} N _{0.019}	0.518	323	62.1	[61]

Note: ^a—The units of B_{theoretical} are m³ CH₄ kg⁻¹ VS. ^b—The units of B_u (experimentally obtained) are m³ CH₄ kg⁻¹ VS. ^c—When digested as a co-substrate at a 20% by weight ratio with another substrate. ^d—Biodegradability (%) = B_u/B_{theoretical} × 100.

The theoretical methane yields of pig, poultry, sheep, and mixed bloods are 0.538, 0.512, 0.446, and 0.518 m³ CH₄ kg⁻¹ VS, respectively (Table 6). Blood biodegradability during AD varies from 48.8 to 93.5% (Table 6) based on the experimental conditions.

Banks and Zhang [52] measured an experimental methane yield of 0.450 m³ CH₄ kg⁻¹ sheep blood VS; this yield was 93.5% of the theoretically calculated methane yield based on the elemental analysis results. Therefore, the biodegradability of the sheep blood during AD is around 93.5%. Similarly, Kim et al. [57] found that pig blood biodegradability is about 82.3% based on the ratio of the ultimate cumulative methane yield to the theoretical methane yield (B_u/B_{theoretical}). In comparison, the degradability of relevant waste was: pig intestine residue (81.7%), pig digestive tract content (70.8%), and cattle rumen content (66.1%) [57].



$$B_{theoretical} = \frac{\left(\frac{c}{2} + \frac{h}{8} - \frac{o}{4} - \frac{3n}{8} - \frac{s}{4} \right) \times 22.4}{12c + h + 16o + 14n + 32s} = \frac{LCH_4}{gVS} \quad (8)$$

Biogas yield of blood (18% DM) ranges between 0.3 and 0.6 m³ kg⁻¹ TS [63,64]. Steffen et al. [65] reported a relatively high value of biogas yield for animal blood (0.65 m³ kg⁻¹ VS for blood of 9.7% TS and VS/TS ratio of 0.95). Blood plasma (20–40% TS) fed as a co-substrate at 10–15% mass ratio produced a biogas yield of 0.40–0.60 m³ kg⁻¹ VS in a biogas plant [65].

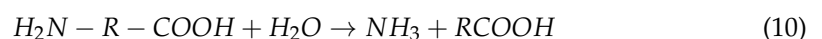
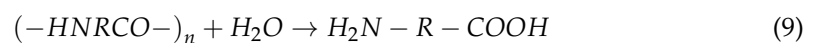
Banks and Wang [29] attributed the low methane yield from ruminal waste to the blood effect; degradation of the blood nitrogen-rich compounds caused the accumulation of high concentrations of ammonia. Banks and Zhang [52] co-digested a mixture of biodegradable municipal waste plus a co-substrate (20% by mass); compared to pig gut with floatation fat, poultry litter, or biodiesel byproduct, sheep blood produced the highest methane content in biogas (58.7%), with a methane yield of 0.357 m³ CH₄ kg⁻¹ VS at STP.

5. Pathways of Protein Degradation

The structure of proteins comprises long chains of amino acids connected by peptide bonds (–CO–NH–). Each amino acid has four active groups attached to its central carbon atom: an amino group (–NH₂), a carboxylic group (–COOH), a hydrogen atom (H), and a fourth group (R) that is specific to each amino acid; depending on the specific group (R), there are 20 amino acids used for protein synthesis [66]. Proteases, which are extracellular enzymes, hydrolyze proteins to free amino acids and polypeptides (Equation (9)). Amino acids are degraded to short- or branched-chain volatile fatty acids (VFAs), nitrogenous compounds (NH₃), phosphates, sulfides (if the amino acid contains the sulfhydryl group),

CO₂, and H₂ [67]. The deamination reaction produces ammonia and VFAs (Equation (10)), and the VFAs are further oxidized to CO₂. In addition, decarboxylation can produce amines (Equation (11)), which have a foul odor [66,68], thus contributing to the offensive odor associated with blood waste.

Two pathways exist for amino acid metabolism: Stickland reactions are needed for the degradation of pairs of amino acids, whereas the degradation of single amino acids requires H₂-utilizing microorganisms, as is the case for carbohydrate fermentation [58]. In the Stickland reaction, one amino acid is used as an energy source and is oxidized, and a second amino acid serves as the electron acceptor [69]. The Stickland reaction is simpler and faster than fermenting uncoupled amino acids [58].



6. Microbiology

Stress factors impose inhibition on microorganisms during AD and work as selectors that promote the dominance of specific bacterial species in the microbial consortium [70,71]. Ammonia is a stress factor during the AD of protein-rich substrates. Frank et al. [72] reported that high ammonia levels resulting from the degradation of protein-rich substrate lower the activity of acetoclastic methanogens, and promote the syntrophic acetate oxidation reaction (i.e., conversion of acetate to H₂ and CO₂) and their conversion to methane by hydrogenotrophic methanogenesis. Anaerobic mixed culture from a biogas plant digesting pig slurry (rich in protein) and maize silage contained proteolytic bacterial species belonging to *Clostridia* [58,73]. Several proteolytic, chemo-organotrophic, and protein-specific anaerobic bacteria species have been isolated from anaerobic digesters: *Proteiniborus ethanoligenes* gen. nov., sp. nov., which belongs to *Clostridia* [74]; *Clostridium thiosulfatireducens* [75]; *Clostridium tunisiense* [76]; and *Proteiniphilum acetatigenes* [77]. The genus *Proteiniborus* is a protein-specific bacteria (they consume only protein). They ferment protein to H₂, acetic acid, ethanol, CO₂, and small concentrations of propionic acid [78]. Recently, Kovács et al. [58] used metagenomic analyses based on next-generation sequencing to monitor the dynamics of the anaerobic microbial culture during pig blood AD; over a period of 12 weeks, the abundance of *Alkaliphilus metalliredigens*, *Alkaliphilus oremlandii*, and *Dethiosulfobivrio peptidovorans* increased by 8.7, 11.4, and 2.0 times, respectively; whereas *Anaerobaculum hydrogeniformans* and *Candidatus Cloacamonas acidaminovorans* disappeared from the culture. Using small subunit rRNA genes and metagenomic sequencing, Frank et al. [72] reported that 5% of the 16S rRNA genes in a commercial, ammonia-tolerant biogas digester was uncultured phylotype (unFirm 1) with metabolic dominance and acetate oxidation capabilities. UnFirm 1 encoded the homoacetogenic-characterizing carbon monoxide dehydrogenase/acetyl coenzyme A (acetyl-CoA) synthase (Acs) operon. They suggested that unFirm 1 is a key to the long-term stability and success of protein-digesting biogas reactors.

Various bacteria such as *Clostridium*, *Vibrio*, *Peptococcus*, *Bacillus*, *Proteus*, and *Bacteroides* secrete extracellular enzymes to hydrolyze proteins into amino acids [79]. Lee et al. [80] quantified the microbial populations through the 16S rRNA genes of bacteria and archaea in protein-fed thermophilic anaerobic digesters using a pyrosequencing analysis and real-time quantitative polymerase chain reaction (QPCR). They observed that the genus *Defluviitoga* and *Keratinibaculum* dominated (10.4% and 8.1%, respectively) the digester. Similar dominance was observed in digesters fed stillage [81] and food waste [82]. *Keratinibaculum paraultunense* is a known protein-consuming bacterium [83].

Immediate effects of ammonia shock, resulting from protein-rich feed, on the transcription and composition of a biogas reactor microbiome were assessed by Fisher et al. [84] using 16S rRNA gene amplicon sequencing. *Clostridia* and *Bacteroidia* were the first and

second most dominant classes in the digester. The abundance of Clostridia decreased from 63% to 40%, while that of Bacteroidia increased from 15% to 23% in the ammonia-treated reactors after 10 days of incubation. They showed that out of the clostridial taxa, MBA08 and the family Caldicoprobacteraceae were the most abundant in the digester.

Digesting blood as a mono-substrate, next-generation-sequencing microbial community analysis showed that the phyla Firmicutes (>99.5% abundance), Proteobacteria, Synergistetes, Actinobacteria, and Bacteroidetes accounted for 99.6% of the sequences [85]. Of the Firmicutes, the orders identified were Bacillales, Lactobacillales, Clostridiales, MBA08, Natranaerobiales, Thermoanaerobacterales, and two (FB2) and three (SC3) unclassified orders [85]. Furthermore, other genera of the Firmicutes have been shown to degrade protein in anaerobic digesters; these include *Anaeromusa*, *Anaerosphaera*, *Aminobacterium*, *Aminomonas*, *Gelria*, *Peptoniphilus*, *Thermanaerovibrio*, *Clostridium*, *Proteiniborus*, and *Sporanaerobacter* [86]. However, members of Bacteroidetes (genera *Fermentimonas* and *Proteiniphilum*), Fusobacteria, and Cloacimonetes can metabolize amino acids in anaerobic digesters [86–88]. Of the Clostridiales order, the genus *Sporanaerobacter sp.* converts blood proteins to VFAs [85]. Despite the phylogenetic diversity of hydrolytic bacteria, the most known species belong to the phyla *Bacteroidetes* and *Firmicutes* [89]. Recently, Xu et al. [90] reported that the dominant phyla in a protein-rich substrate fed into an anaerobic digester were Bacteroidetes, Firmicutes, Chloroflexi, and Proteobacteria.

7. Previous Studies on Blood Anaerobic Digestion

Generally, AD of blood-containing waste was stigmatized by the low biogas and methane yields reported in the literature, lengthy hydraulic retention time (HRT) required, and low organic loading rate (OLR). The latter two operational parameters increase the volume of the digester, which translates into increased capital and operation costs. Table 7 includes reported results on blood AD either as a sole substrate or as a co-substrate in batch and continuous reactors.

Several types of reactors have been used to investigate the AD of blood (see Table 7). These include batch, semi-continuous, and continuous reactors. The reactor volumes were 2–47 L (batch), 1–4000 L (continuous), and 2–3600 L (semi-continuous). Batch reactors were operated under normal and sub-atmospheric pressures. Blood was digested in different types of continuous and semi-continuous reactors, such as an upflow anaerobic sludge blanket reactor (UASBR), an upflow anaerobic filter reactor (UAFR), a completely stirred tank reactor (CSTR), and two-stage reactors. Some studies used reactors filled with culture-immobilizing materials such as biochar and bamboo cylinders. Only one study reported data from an actual 7600 m³ biogas plant fed with manure and blood (83% and 17% by mass) [82].

Ozturk [91] investigated AD of blood as a mono-substrate in semi-continuous 3.6 m³ working volume continuously mixed digester operated at 35 °C with an HRT of 18 days. No methane was observed, but a strong offensive odor was produced; therefore, it was concluded that AD of blood alone is difficult, and feeding another organic waste as a co-digestate is suggested. Similarly, Cuetos et al. [54] reported that digesting poultry blood in batch yields 0.047 m³ biogas kg⁻¹ VS; this is a very low yield compared to the theoretical methane potential (Table 6), and was accompanied by high pH (8.8) and high ammonium levels (4500 mg L⁻¹) during the entire experiment. The average concentration of free ammonia was 1813 mg L⁻¹, which inhibited the culture. Nevertheless, Kovács et al. [58] reported a specific methane yield of 0.447 m³ kg⁻¹ protein dry matter from digesting precipitated blood protein in a fed-batch reactor at 37 °C and 56 days. This yield is close to the theoretical yield (Table 6). These researchers emphasized that sustained biogas production from blood protein is achievable, given that the microbial community is well characterized, understood, and controlled.

Bauer [92] used dilution to decrease the strength of blood. By digesting blood water from an abattoir in a batch reactor (35 °C) fed 0.6 g VS L⁻¹, a specific biogas yield of 0.733 m³ kg⁻¹ VS and a specific methane yield of 0.117 m³ kg⁻¹ VS within 10 days of

incubation were obtained [92]. Although the methane yield was still low compared to the theoretical yield of methane from the blood (Table 6), dilution with water enhanced the yield. Alternatively, Cuetos et al. [54] used co-digestion to overcome the ammonia inhibition observed during the digestion of blood as a mono-substrate. Poultry blood and maize residues (30% and 70% by mass of VS, respectively) were mixed to dilute the nitrogen, and yielded $0.188 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ in batch assay at $34 \text{ }^\circ\text{C}$. However, a semi-continuous reactor produced only $0.065 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ due to the accumulation of VFAs and non-degraded materials [54].

Integrating the concepts of co-digestion and dilution, Ozturk [91] co-digested cattle blood:cattle manure:water (30:20:50 mass ratio) in a semi-continuous 3.6 m^3 working volume continuously mixed digester operated at $35 \text{ }^\circ\text{C}$ with an HRT of 18 days. They reported a biogas production rate of $0.64 \text{ L min}^{-1} \text{ m}^{-3}$ with a COD removal of 34% in a 30-day study.

Hansen and West [49] obtained $0.14 \text{ m}^3 \text{ biogas m}^{-3} \text{ digester}$ by co-digesting blood (2% by weight) and rendering plant waste condensate (98% by weight) at $\text{OLR } 1.0 \text{ kg COD m}^{-3} \text{ d}^{-1}$ in an upflow anaerobic sludge blanket (UASB) reactor at $35 \text{ }^\circ\text{C}$ with an HRT of 15.6 days. A blood: wastewater (1:49 *w/w*) mixture was co-digested by Marcos et al. [93] in a discontinuous digester operated at $38 \text{ }^\circ\text{C}$. They achieved 56.9% COD removal at an $\text{OLR } 0.17 \text{ kg COD m}^{-3} \text{ d}^{-1}$. Cuetos et al. [13] co-digested poultry blood with the organic fraction of municipal solid waste (OFMSW) to avoid the inhibition due to the accumulation of ammonia content in a semi-continuous anaerobic digester operated at $32 \text{ }^\circ\text{C}$. They reported specific methane yields of $0.33 \text{ m}^3 \text{ kg}^{-1} \text{ VSS}_{\text{fed}}$, a methane production rate of $0.5 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$, and 60% CH_4 in biogas at an $\text{OLR of } 1.5 \text{ VSS}_{\text{fed}} \text{ m}^{-3} \text{ d}^{-1}$ with an HRT of 36 days. Increasing the OLR to $2.0 \text{ kg VSS}_{\text{fed}} \text{ m}^{-3} \text{ d}^{-1}$ destabilized the process in a short time, and lowered the specific methane yield to $0.20 \text{ m}^3 \text{ kg}^{-1} \text{ VSS}_{\text{fed}}$. In a continuous reactor, the methane yield for blood-containing mixed abattoir waste increased to $0.33 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$ compared to $0.117 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$ in batch [92].

Table 7. Comparative performance of blood anaerobic digestion.

Substrate	Reactor Configuration	Temp. (°C)	HRT (d)	OLR	SMY (m ³ kg ⁻¹ VS) Unless Indicated Otherwise	Reference
Bovine blood and water, ruminal bovine content, and beef manure	Batch	39	6	Bovine blood and water (70% bw), ruminal bovine content (20% bw), and beef manure (10% bw)	507 L kg ⁻¹	[94]
Poultry blood assisted with activated carbon	Batch Semi-continuous	37	20	Blood:activated carbon ratio of 1.5 kg COD m ⁻³ d ⁻¹	0.317 0.216 L kg ⁻¹ COD _{fed}	[95]
Poultry blood and poultry wastewater	Semi-continuous biochar filled (50% volume) UAFR	34–38	9.2 6.9 3.7 3.0	2.9 4.7 3.9 4.6 kg COD m ⁻³ d ⁻¹	96.2 81.6 81.5 46.5 L kg ⁻¹ COD _{fed}	[2]
Poultry blood and poultry wastewater	Semi-continuous bamboo cylinders filled (50% volume) UAFR	34–38	27.7 13.7 9.2 6.9 3.7 3.0	11 1.8 2.9 4.7 3.9 4.6	25 17.5 13.6 24 11 10.3	[2]
Blood and manure	semi-continuous 4 m ³ CSTR	35	18	Blood:manure:water mass ratio of 30:20:50	0.55 m ³ d ⁻¹ m ⁻³	[91]
Precipitated pig blood protein	Fed-batch (5 L)	37	56	20 g of protein DM	0.447 m ³ kg ⁻¹ protein DM	[58]
Poultry blood and maize residues	CSTR (3 L)	34	36	3.1 kg ⁻¹ VS m ⁻³ d ⁻¹	0.188	[54]
Blood (20%) and municipal solid waste	Continuous CSTR (5 l)	36	30	2 kg VS m ⁻³ d ⁻¹ 3 kg VS m ⁻³ d ⁻¹	0.357	[50]
Bauer	Batch	35	20	0.6 kg VS m ⁻³ kg VSS _{feed} m ⁻³ d ⁻¹	0.117	[92]
Poultry blood and OFMSW	Semi-continuous	32	36	1.5 2.0	0.33 0.20	[13]
Pig blood	Batch	55	30–35	Blood concentration: 5% 20% 50% 100%	m ³ kg ⁻¹ blood: 0.56 0.43 0.12 0.056	[14]
Blood 17% (by mass) and manure	7600 m ³ Actual biogas plant	53	17–18	Blood loading: 0.16 kg N m ⁻³ day ⁻¹	32% lower biogas than manure only	[96]
Blood	sub-atmospheric pressure laboratory scale digester	32		0.08–1.61 kg COD kg ⁻¹ culture dry matter		[31]
Poultry blood and ruminal content (1:10 dw)			20	Co-substrates TS = 3–4%	1.0 m ³ biogas m ⁻³ d ⁻¹	[97]

Table 7. Cont.

Substrate	Reactor Configuration	Temp. (°C)	HRT (d)	OLR	SMY (m ³ kg ⁻¹ VS) Unless Indicated Otherwise	Reference
SCM and SCSSW	Batch (2 L)	35	30	SCM (10% bw) and SCSSW (11% bw) SCSSW (21% bw)	0.29 0.11	[51]
Poultry blood, bone and trimmings, offal and feather	CSTR	31	13–100	0.5–2.1 kg VS m ⁻³ d ⁻¹	0.09–0.55	[15]
Solid slaughter-house waste	CSTR, 2 L	35	50	0.8 kg VS m ⁻³ d ⁻¹	0.52–0.55 0.55–0.67	[98]
Blood (16%) and solid poultry slaughterhouse waste	Batch CSTR		50–100	0.8 kg VS m ⁻³ d ⁻¹	0.52–0.55	[98]
Cattle blood and rumen paunch (gut fill) (1:3 by weight)	Two-stage hydraulic flush reactor (4 L)-CSTR (1 L) with immobilization	35	2–10	3.6 kg TS m ⁻³ d ⁻¹ 0.58–7.0 kg COD m ⁻³ d ⁻¹	0.12–0.25 m ³ kg ⁻¹ TS	[99]
Cattle and sheep paunch, blood, and wastewater	CSTR, 105 m ³	NR	43	0.36 kg COD m ⁻³ d ⁻¹	0.18 m ³ kg ⁻¹ COD	[36]
Blood (16%) and solid poultry slaughterhouse waste	Batch	35			0.55–0.67	[98]
Slaughterhouse waste, manure, fruit and vegetable waste	CSTR	35	50–100	0.8 kg VS m ⁻³ d ⁻¹	0.52–0.55	
Blood (5.7%)	Semi-continuous CSTR (2 L)	35	30		0.35	[60]
Poultry slaughterhouse waste 15.8%	Semi-continuous CSTR (3 L)	31	50	1 kg VS m ⁻³ 0.5 kg VS m ⁻³	0.55	[28]
Blood (5%) Poultry litter and pig manure	Batch (17 L)	36	30		0.12	[100]
Blood (2%) and rendering plant condensate (98%)	UASB	35	15.6	1.0 kg COD m ⁻³ d ⁻¹	0.14 m ³ kg ⁻¹ COD	[49]

Note: Temp.: temperature; HRT: hydraulic retention time; OLR: organic loading rate; SMY: specific methane yield; bw: by weight; dw: dry weight basis; CSTR: continuously stirred tank reactor; OFMSW: organic fraction of municipal solid waste; SCM: swine and cattle manure; SCSSW: swine and cattle slaughterhouse solid waste (consists of rumen cow (57.1% bw), stomach content and gut fill of swine (9.4), blood cow (28.6), and blood swine (4.9)); UAFR: upflow anaerobic filter reactor; UASB: upflow anaerobic sludge blanket.

Banks [36] reported a specific methane yield of $0.18 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ COD}_{\text{fed}}$ from AD of cattle and lamb paunch contents, blood, and process wastewaters applied at an OLR of $0.36 \text{ kg COD m}^{-3} \text{ d}^{-1}$ with an HRT of 43 days. This low yield was affected by fluctuations and an overload of blood in the feed, which destabilized the process through ammonia inhibition. Alvarez et al. [51] digested different proportions of mixed swine and cattle manure (SCSM), swine and cattle slaughterhouse solid waste (SCSSW), and fruit and vegetable waste in a batch reactor at $35 \text{ }^\circ\text{C}$ with an HRT of 30 days. The SCSSW contained (% by weight) cow rumen (57.1), stomach content and gut fill of swine (9.4), cow blood (28.6), and swine blood (4.9). Alvarez et al. [51] obtained a maximum methane yield of $0.29 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$ from a mixture of SCSM and SCSSW (10% and 11% by weight, respectively); VS reduction was 75%, while the pH increased from 6.6 to 8.8 by the end of the experiment. Digesting SCSSW (21% by weight) alone yielded only $0.11 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$ with 35% VS reduction; the authors attributed this inhibition to the accumulation of ammonia due to the nitrogen-rich substrate [51]. The researchers noticed that adding fruit and vegetable waste at 17% by weight inhibited methane production and decreased its yield by 90%. Banks and Wang [99] observed almost the same inhibition in a continuous reactor digesting mixed swine and cattle manure and cattle blood (1 part by weight) and gut fill (3 parts gut fills by weight) with a similar methane yield of $0.17 \text{ m}^3 \text{ kg}^{-1} \text{ TS}$ and 50% reduction in TS during 30 days of HRT. Lopez et al. [97] co-digested poultry blood (10% dry weight basis) with ruminal content (90%) at TS of 3–4% and an HRT of 20 days and measured a biogas production rate of $1.0 \text{ m}^3 \text{ biogas m}^{-3} \text{ d}^{-1}$. It is evident that the previously discussed studies demonstrated that the AD of blood as a mono-substrate in the batch was successful in one study only [54], but with a low biogas yield ($0.047 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$). Dilution improved the methane yield in batch and continuous reactors, with a specific methane yield of 0.117 to $0.33 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$ [92]. Co-digestion of blood with other substrates resulted in a specific methane yield of 0.11 to $0.18 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$, with decreasing yield as the proportion of blood increased in the substrate.

Interestingly, Salminen et al. [98] reported a specific methane yield of $0.5\text{--}0.7 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$ from co-digesting poultry blood, meat, and bone trimmings. A maximum of $0.67 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$ was reported for batch AD of a mixture (weight ratio) of solid poultry slaughterhouse waste of bone and trimmings (42%), blood (16%), offal (32%), and feather (10%) [98]. Reategui et al. [94] digested a mixture (% by weight) of beef manure (10%), bovine ruminal content (20%), bovine blood, and water (70%) in a batch digester at $39 \text{ }^\circ\text{C}$; they reported a biogas yield of $0.507 \text{ m}^3 \text{ kg}^{-1}$ of the substrate mixture within 6 days of incubation time. They concluded that the high concentration of nitrogen in the blood and rumen content stabilizes the pH, and that conversion of the VFAs (isobutyric and valeric acids) increases the biogas yield. The results reported by Salminen et al. [98] and Reategui et al. [94], when compared to the results obtained from other studies (Table 7) for co-digestion of blood-containing substrate, call for more insights into the mixture of the co-substrates.

While the previous studies indicated the difficulty of digesting blood as a mono-substrate, they provided evidence that co-digestion is a strategic solution; however, the co-digestate has to be selected carefully to avoid imbalance in the process. The studies that investigated blood co-digestion based on the selection of the co-digestate mostly considered a C:N ratio within the optimum range for AD. However, no insight was provided into how the co-digestates would affect the evolution of free ammonia and VFAs in the reactor. Moreover, the accumulation of non-biodegradable byproducts also requires precise attention during the selection of a co-digestate.

8. Issues Faced during Anaerobic Digestion of Livestock Blood

There are about 8000 biogas plants in the EU. Still, none of them digests a protein-rich waste as a mono-substrate despite the fact that this type of waste is produced continuously in large quantities (around 10^6 metric tonnes year⁻¹ from pigs, cattle, and poultry slaughtering worldwide) [58]. This indicates that there are issues associated with the AD of

this type of waste. The EC-Regulation (EC) No. 1774/2002 classifies the blood of animals slaughtered in a slaughterhouse under Category 3, Animal byproducts (ABPs), and are considered fit for human consumption. Thus, blood should be pasteurized and hygienized before feeding it to a biogas process [101]. This increases the cost of AD and decreases the net energy yield.

Anaerobic digestion of blood faces operational issues such as overloading and inhibition. Slaughterhouse blood is rich in proteins (nitrogen-rich compounds); its carbon:nitrogen (C:N) ratio is low compared to other raw biomasses (see Table 8). It contains 81.25–197.3 g proteins kg⁻¹ (Table 2). Protein degrades to release nitrogenous compounds, which inhibit the microorganisms carrying out the bioreactions in AD.

Degradation of proteins during hydrolysis, as well as acidogenic and acetogenic fermentation steps, releases free (unionized) NH₃-N (FAN) and ionized ammonium N (NH₄), together called total ammonia nitrogen (TAN). Inhibition of AD is related to TAN and FAN levels [102]. High levels of total ammonia (NH₃ and NH₄⁺) are observed during blood AD. Although optimal concentrations of ammonia (NH₃) provide sufficient buffer capacity to the AD medium [13], high concentrations of NH₃ inhibit the microbes mediating the AD bioreactions [103]. Cuetos et al. [95] reported that free ammonia and ammonium concentrations were 1813 mg L⁻¹ and 4500 mg L⁻¹, respectively, during AD of residual poultry blood in a batch reactor at 37 °C with an inoculum-to-substrate ratio of 1:2. This free ammonia level imposed severe inhibition, which caused accumulation of VFAs (acetic acid = 2180 mg L⁻¹, C3–C5 about 350 mg L⁻¹ each).

Variations in substrates, inocula, temperature, pH, reactor configuration and operation mode, and adaptation result in the wide variation of inhibitory NH₃ levels (a 1500–7000 mg TAN L⁻¹) as observed and reported in the literature [14,103]. Methanogens are mainly inhibited by FAN under high nitrogen concentration [104] with FAN in solution, rather than NH₄ causing the inhibition [105].

Table 8. Carbon-to-nitrogen ratio of various biomasses.

Substrate	Nitrogen Content (%mass) Unless Indicated Otherwise	C:N Ratio	Reference
Urine	15–18	0.8	[106]
Slaughterhouse waste	7–10	2	[106]
Blood	10–14	3	[106]
Poultry blood	12% Dry base	2.8	[54]
Precipitated pig blood	52.7 g kg ⁻¹	3.2	[58]
Poultry carcass		4	[106]
Cattle carcass		10	[106]
Sewage		11	[106]
Vinasse		12	[107]
Swine carcass		14	[106]
Manure			
Chicken	3.2	7–10	[106] [108]
Swine		12–16	[109]
Poultry	6.3	15	[106]
Sheep		16	[106]
Cow paunch		17–21	[110]
Cow	1.7	18–20	[106]
Dairy		22	[108]
Steer	1.35	25.3	[106]
Horse	2.3	25–30	[106]
Corn stover		50–63	[111]
Straw		83	[107]
Bagasse		116	[107]
Corn cobs		123	[112]
Wheat straw	0.3	50–128	[111]
Rice straw		52	[108]
Saw dust	11	511	[106]
Wood chip	0.06	767	[106]

Temperature affects NH_3 dissociation and its concentrations in solution. Anaerobic digestion can be conducted at psychrophilic, mesophilic, or thermophilic temperatures. The TAN concentration increased six times by increasing the temperature from 37 to 55 °C [113]. Thus, low temperature is suggested to control NH_3 inhibition during AD of livestock blood.

Anaerobic digestion of poultry blood and poultry wastewater (PBPW) was inhibited at high TAN and VFA concentrations; Wang [2] measured TAN and VFA concentrations of 3.1 g L⁻¹ and 15.25 g L⁻¹, respectively. Although this concentration of TAN enhanced the buffering capacity and maintained a pH of 6.6–7.7, it was inhibitory to methanogens. Diluting the substrate reduced TAN and VFAs concentrations, but it did not improve methane yield [2].

Nielsen and Angelidaki [96] reported feeding an actual biogas plant (capacity 7600 m³, operated thermophilically at 53 °C with an HRT of 17–18 days) 437 metric tonnes day⁻¹ of feedstock composed of manure (83% by mass) and blood (17% by mass). They concluded that the blood imbalanced the plant performance and lowered the biogas production by 32% by increasing ammonia and VFAs levels. Ammonia concentrations increased immediately, while VFAs accumulated over about 2.5 the HRT (i.e., 45 days). Upon discontinuing blood feeding, the reactor recovered and resumed its original methane yield with almost one HRT [96]. This actual incidence was further simulated in the laboratory using mesophilic and thermophilic reactors. The VFA concentrations increased significantly in the thermophilic reactor immediately after feeding blood (equivalent to 0.16 g N L⁻¹ day⁻¹). In comparison, only a moderate increase in the VFA concentrations was observed in a mesophilic reactor, which also showed an increased methane yield because the methane potential of blood is greater than that of the cattle manure replaced [96]. The thermophilically reactor showed a lower methane yield six days after blood feeding was started. Despite that blood feeding was stopped, the methane production did not recover until the end of the experiment [96]. The mesophilic reactor showed signs of inhibition, and a decrease in the methanogenesis two weeks after feeding the blood was started; however, its free NH_3 concentration was less than the inhibitory concentration (0.7–1.0 g N L⁻¹) suggested by Angelidaki and Ahring [114] and Hansen et al. [115]. Nielsen and Angelidaki [96] found that both free NH_3 and some of the blood components might destabilize the reactor, and the operation temperature strongly impacted the stability of manure and blood co-digestion. They concluded that co-digestion of blood and manure is not possible at thermophilic or mesophilic temperatures when the blood is 17% by weight. Therefore, they recommended that blood should be fed at a smaller percentage to avoid inhibition [96].

Another issue is the production of sulfides upon the degradation of sulfur-containing proteins. Sulfides are toxic, corrosive, and generate a strong unpleasant odor [91] during the AD of blood. The odor is attributed mainly to hydrogen sulfide (H_2S), which has the odor of rotten eggs. In addition, the presence of sulfur allows sulfate-reducing bacteria (SRB) to divert the electron equivalents from methane-forming pathways to H_2S -forming pathways. The SRBs are well-known strong competitors to methanogens. Around 0.7 L of methane is lost for each gram of sulfide formed [62]. Generally, methanogenic cultures grow slowly (the doubling time is four days at 35 °C) [62].

Furthermore, the percentage of the substrate's (i.e., protein) electron equivalent that is channeled to synthesizing new microbial biomass is only 8% for protein compared to 28% for carbohydrates [62,116,117]. This means fewer new microorganisms cells are formed during the AD of proteins. A methanogenic microbial culture grows slowly. When it is under the toxicity of ammonia and sulfides, it will be vulnerable to upsets, and its recovery is difficult. The difficulty also arises because the SRBs grow faster than methanogens.

Some SRBs produce acetate through fermentative and proton-reducing reactions in addition to sulfate reduction [118,119]. Therefore, they are more flexible and tolerant to changes within the reactor than the fastidious methanogens. Examples of the acetate-forming SRBs microorganisms include *Desulfomicrobium*, *Desulfobulbus*, and *Desulfobotulus* [120]. Moreover, SRB groups such as *Desulfococcus*, *Desulfosarcina*, and *Desulfobotulus* are

very competitive for H₂ when sulfate is absent [118]. Thus, they deprive hydrogenotrophic methanogens of hydrogen and divert it away from methane formation.

9. Strategies Attempted for Blood Anaerobic Digestion

Several strategies have been attempted to alleviate and control the inhibition imposed by the byproducts of blood protein degradation. Acclimating the microorganisms to high NH₃ levels, optimizing the C:N ratio, controlling pH, and low temperature are strategies to control the NH₃ inhibition [111,113].

Cuetos et al. [95] investigated the influence of adding activated carbon at mass ratios (blood:activated carbon) of 1.5, 3.0, and 4.5. They found that at a minimum ratio of 1.5 alleviated the inhibition and increased the specific methane yield (l kg⁻¹ VS) from 46.5 to 317.4 (batch reactor) and 216 (semi-continuous reactor). However, an analysis revealed that such an application would be economically unfavorable at an industrial scale due to the high price of activated carbon [95].

Protein adsorption by activated carbon increases with increasing temperature [121]; this might provide a potential solution to blood digestion in thermophilic operation. Increasing the quantity of activated carbon added increases the protein adsorption because it increases the surface area, leading to less protein degradation and thus reducing the amount of ammonia present to inhibit the microorganisms. Based on an irregular pattern of the observed activated carbon–acetic acid affinity with observed low adsorptive capacity, Cuetos et al. [95] excluded VFAs' adsorption to the activated carbon as a factor in the observed improvement of blood AD. Instead, they suggested that favoring microbial metabolism might be a factor. The detailed understanding of the adsorbent's effects on AD needs further research investigations, and the mechanisms responsible for the improvements reported should be illustrated.

Diluting the nitrogen-rich substrate has been used to alleviate TAN inhibitory effects on methanogens. Wang [2] used a biochar-filled anaerobic filter (BFAF) to digest a mixture of poultry blood and poultry wastewater (PBPW); the biochar was used to dilute the substrate. It was concluded that diluting the nitrogenous substrates improved the COD removal slightly at moderate OLRs; however, this improvement was lost at high OLR and short HRT values [2]. Biochar's porous structure might be behind this improvement, because it could have protected some methanogens from the TAN-imposed inhibition. Relevant to such a speculated mechanism, using granular microbial culture might provide such a protection as well. Culture granulation has been recognized as a strategy to manage the inhibition imposed by, for example, long-chain fatty acids. Recently, Xu et al. [90] found that the effect of sewage-sludge-derived biochar on the AD of protein-rich substrate was minor compared to that observed on carbohydrate- and lipid-rich substrates. It was found that biochar does not enhance the growth of methanogens in protein-rich-substrate AD.

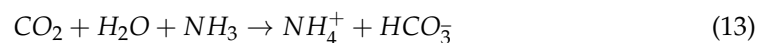
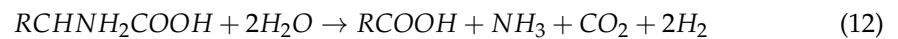
Following the adaptation of the culture, Kovács et al. [58] digested precipitated pig blood protein as a model protein-rich substrate in a laboratory-scale fed-batch mesophilic bioreactor, and reported a specific methane yield of 0.447 m³ CH₄ kg⁻¹ protein DM. This is a relatively high yield for blood as a mono-substrate compared to the values cited in Tables 6 and 7; however, it compares well with the yield reported by Kim et al. [57] for pig blood (0.443 m³ CH₄ kg⁻¹ VS) and translates into 83% biodegradability. Ammonia and H₂S accumulation limited the degradation of blood protein, and might have decreased the methane yield. However, Kovács et al. [58] suggested that the reported methane yield could be achieved and sustained if the system is controlled based on a proper characterization and detailed understanding of its microbial community and its metabolic activities.

Jędrzejewska et al. [31] investigated the impact of reduced pressure (vacuum) on methane production from blood in a laboratory-scale digester at 32 °C. Compared to a control digester (maintained at atmospheric pressure), a digester operated at sub-atmospheric pressure maintained at an average pH of 7.92 irrespective of the OLR applied. The pH of the control digester decreased systematically with the accumulation of organics, and dropped

to 5.53 within 30 days of the experiment. The reduced headspace pressure increased the percentage of CH₄ in biogas by 5–15%, NH₃ by 3–4%, and H₂S by 1.0% [31].

Banks and Wang [99] used two-stage anaerobic digesters (a hydraulic flushing hydrolyzer followed by a completely mixed immobilized-cell digester) and successfully controlled the inhibition imposed by NH₃ and VFAs accumulated during the digestion of cattle blood and cattle rumen. The first stage provided a short liquid retention time but a long solid retention time, and reduced solids by 87% compared to only a 50% reduction in a single-stage digester (the same retention time for liquid and solids). At an HRT of 2 and 10 days and OLR 0.58–7.0 kg COD m⁻³ d⁻¹, the second-stage digester removed 65–78% of the COD and produced a specific methane yield of 0.12–0.25 m³ CH₄ kg⁻¹ COD removed [99]; the upper limit of the methane yield was low, given that it was reported per COD removed. Acclimating the microorganisms to high NH₃ levels, optimizing the C:N ratio, controlling pH, and low temperature are strategies to control NH₃ inhibition [111,113].

The AD of proteins such as blood is characterized by increased alkalinity. Blood protein releases free NH₃, which forms ammonium bicarbonate when it reacts with CO₂ released by the anaerobic bioreactions (Equations (12) and (13)); ammonium bicarbonate increases the alkalinity [122,123]. Cuetos et al. [13] observed that blood contributes to the buffering capacity when digested with a carbohydrate-rich substrate such as OFMSW.



Blood is very rich in protein and its nitrogenous content is high; thus, it should be co-digested with other substrates of high carbon content to adjust the carbon: nitrogen ratio (C:N) within the optimum range for AD. Feedstock such as manure, wheat straw, and paunch could be co-digested with blood. Blood provides powerful and nutritive components to anaerobic microbial cultures.

10. Kinetic of Biogas Production from Livestock Blood Anaerobic Digestion

Few studies have discussed the kinetics of blood AD; however, the kinetics of proteins have been studied extensively. Some research investigations reported the first-order reaction rate constant of protein hydrolysis during AD, and two studies reported the same value for the entire anaerobic bioconversion of blood water to methane (see Table 9). The first-order rate constant (*k*) for blood water was 0.29 d⁻¹ compared to glucose (0.46 d⁻¹), whereas the methane percentage in biogas ranged between 17% and a maximum of 21% [92].

Incubating pig blood at a 5% concentration with 60% inoculum has been reported as optimal in batch studies conducted at 55 °C with a methane yield of 561 L CH₄ kg⁻¹ pig blood. This methane yield was comparable to the theoretical yield [14]. Methane production from blood had a short lag phase of 3–5 days, but required 30–35 days to complete. Dilution of pig blood increased its methane yield; the best results were associated with the highest dilutions [14]. Compared to the methane yield obtained from feeding blood at a concentration of 5%, when feeding blood at concentrations of 20%, 50%, and 100%, the methane yield was 76%, 21%, and 10%, respectively. At blood concentrations of 5% and 20%, about 90% of the total methane yield was obtained within 8 days; this was a relatively short time compared to other substrates [14]. Among meat and bone flour, fat, blood, hair, meat, and ribs, the raw waste of pig slaughterhouse blood has the lowest fat content, but the highest protein content. This high protein content of blood makes it amenable to ammonia inhibition, particularly in thermophilic conditions, which could worsen with increased ammonia-N load [14].

Table 9. The first-order reaction rate constant of anaerobic digestion of proteins.

Substrate	Temperature (°C)	First-Order Reaction Rate Constant (d ⁻¹)	Reference
Protein		0.0018–0.0197 ^b	[124]
Bloodwater	55	0.29 ^b	[14]
Bloodwater		0.29 ^b	[92]
Proteins		0.02–0.03 ^a	[125]
Proteins	35	0.01–0.1 ^a	[126]
Proteins	55	0.015–0.075 ^a	[127]
Proteins		0.2–0.4 ^a	[128]
Proteins		0.25–0.28 ^a	[129]
Proteins	55	0.65 ^a	[130]
Albumin	37	0.57 ^b	[131]
Alanine	37	0.92 ^b	[131]
Casine	37	0.35 ^b	[131]
Gelatine	37	0.60 ^b	[131]
Glycine	35	0.44–0.98 ^b	[132]
Glycine	37	0.19 ^b	[131]
Glycine	35	0.10 ^b	[133]
Leucine	35	0.31–0.63 ^b	[132]
Leucine	37	0.13 ^b	[131]

Note: ^a—hydrolysis; ^b—entire biogas production.

11. Remarks and Potential Future Research

Few studies have investigated the microbiology of blood's AD to elucidate the functionality of various trophic groups of microorganisms, the mechanisms that affect their dynamics, and eventually their dominance. Accordingly, advanced molecular biology methods need to be integrated with engineering reactor studies of blood AD.

The kinetics of blood's AD bioreactions (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) requires further studies and quantification. Particularly, the kinetics of the enzymes mediating these bioreactions and the associated inhibition need to be investigated.

No studies exist for blood AD at low temperatures, although previous studies showed that it could be a strategy to manage the inhibition imposed by the nitrogenous compounds released during blood's protein degradation.

Further studies are also required to illustrate and decipher the mechanisms by which adsorbents improve the methane yield and reduce the inhibition during blood AD. The kinetics of blood AD under the effect of an adsorbent needs to be quantified.

12. Conclusions

Although difficult, anaerobic digestion (AD) of blood as a mono-substrate is possible with careful system control based on effective characterization and proper understanding of the microbial community and its metabolic activities. Accordingly, a specific methane yield of 0.45 m³ kg⁻¹ blood protein has been achieved. Co-digestion of blood and other feedstock proceeds well when the mixture is well designed. So far, the specific methane yield from co-digesting blood and other substrates ranges between 0.1 and 0.7 m³ kg⁻¹ VS. Further research is required on microbiology and kinetics, the role of adsorbents and how they affect the dynamics of the microbial community, reactor configurations and operation, and culture adaptation during AD of blood for better control/management.

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