

Review

Advances in Biorefinery of Cattle Manure for Value-Added Products

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Abstract: A large amount of cattle manure is generated in the US every year, creating challenges for ecosystem sustainability while offering opportunities for value-added products through biorefinery. While methods like land application, composting, and anaerobic digestion are well established for treating cattle manure, advanced biorefinery technologies are needed to enhance the utilization of cattle manure or its digestate. Based on the composition of cattle manure, this review discusses technologies for the biorefinery of cattle manure or its digestate, including the recovery of fiber and protein, and the production of platform chemicals, such as volatile fatty acids, lactic acid, medium-chain carboxylic acids, ethanol, single-cell protein, and lipids. Additionally, this review discusses process integration for simultaneously producing multiple value-added products from cattle manure using various biorefinery technologies. By offering insights into different approaches, this review aims to support the development of integrated biorefinery processes, thereby improving the economic and environmental sustainability of cattle manure management.

Keywords: manure management; biorefinery; fiber; protein; platform chemicals; fermentation; anaerobic digestion



Citation: Chuenchart, W.; Timilsina, A.P.; Ge, J.; Shah, A. Advances in Biorefinery of Cattle Manure for Value-Added Products. *Fermentation* **2024**, *10*, 568. <https://doi.org/10.3390/fermentation10110568>

Academic Editor: Bartłomiej Zieniuk

Received: 4 October 2024

Revised: 1 November 2024

Accepted: 5 November 2024

Published: 7 November 2024



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1. Introduction

The US has been recognized as the world's largest beef producer (12.7 million metric tons) and the third largest milk producer (102.0 thousand metric tons) in 2021 [1,2]. Globally, 942.63 million cattle are estimated to generate 3.4 billion dry metric tons of manure annually [3]. About 43.4 million metric tons of cattle manure is produced in the US annually, including 24.4 million metric tons from beef cattle and 19.0 million metric tons from dairy farming [4]. Cattle manure is a complex organic substance containing organic matter, ruminal bacteria, and nutrients, which have significant impacts on the environment and human or animal health if not managed properly. Cattle farming primarily contributed to 3.8 billion metric tons of CO₂-equivalent greenhouse gas (GHG) production, accounting for 62% of all livestock emissions in 2015, due to both enteric fermentation and manure left in the pasture [5]. In addition, cattle manure harbors a variety of viruses, bacteria, and protozoa that could be pathogenic to humans and animals [6]. Conventional cattle manure management methods, such as land application and aerobic composting, are usually associated with concerns of GHG emissions and/or insect vectors (Figure 1). Anaerobic digestion (AD) is a widely recognized method for managing cattle manure, generating biogas (composed of approximately 60% methane and 40% CO₂) for bioenergy, along with digestate, which contains undigested organic matters and is typically handled through land application (Figure 1). AD under thermophilic conditions could deactivate *Escherichia coli* and *Salmonella* [7,8], yet higher VFAs and ammonia conditions could also contribute to

pathogen inactivation [7,9]. Additionally, further treatment (i.e., thermal, aerobic) could also be applied for pathogen removal before land application [6]. Thermal conversion processes, such as pyrolysis and gasification, have also been studied for treating cattle manure and generating products such as biochar, bio-oil, and syngas [10–12].

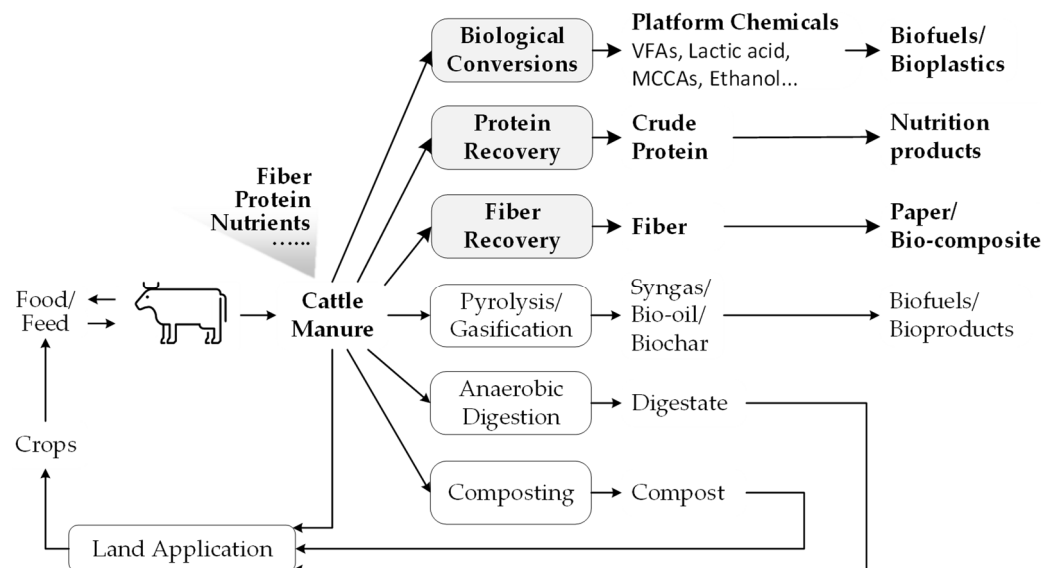


Figure 1. Different approaches for management of cattle manure.

Biorefinery has been recognized as a promising approach for producing value-added products from waste streams through various processes, such as thermochemical treatment, liquid/solid separation, and bioconversion [13]. GHG emissions from cattle manure or cattle manure digestate are expected to be reduced through the biorefinery process, as organic matter is utilized rather than being disposed of into the environment. Moreover, biorefinery can reduce pathogen transmission by managing cattle manure under controlled conditions. As a result, biorefinery holds great potential to enhance both the economic and environmental sustainability of cattle manure management.

Fiber and crude protein are the two major components of cattle manure or cattle manure digestate and can potentially be recovered for beneficial use (Figure 1). Furthermore, cattle manure or cattle manure digestate can be used as a sole feedstock or co-feedstock for producing platform chemicals, such as volatile fatty acids (VFAs), lactic acid, medium-chain carboxylic acids (MCCAs), and ethanol [13,14]. These platform chemicals can be further used for high-value products of biofuels and biomaterials [13]. Recently, the platform chemical biorefinery has also emerged as a novel valorization approach due to advances in bioprocess and scale-up, which makes it more compatible with downstream processes of existing petroleum refineries (Figure 1) [13,14]. Despite several studies on platform chemicals from cattle manure or cattle manure digestate, there remains no critical or comprehensive review of this subject.

This article discusses the composition of cattle manure, highlighting its potential for biorefinery, then reviews fractionation methods for fiber and protein recovery from cattle manure or cattle manure digestate, and finally provides a comprehensive review of platform chemical production using cattle manure as a mono- or co-substrate. To holistically manage cattle manure, lipid production from oleaginous microorganisms based on cattle manure digestate was also included.

2. Composition of Cattle Manure

The composition of dairy and beef manure is presented in Table 1. Overall, the two types of cattle manure have similar compositions. It should be noted that the numbers in Table 1 are for fresh manure without blending with bedding materials, such as sand,

straw, and/or sawdust. The total solids (TS) of fresh cattle manure are about 15%. Most (around 85%) of the mass in fresh cattle manure is water, making it a “wet” stream that is challenging for direct treatments with high temperatures, such as pyrolysis, gasification, and incineration. Most (around 85%) of the dry matter are volatile solids (VS) or organic matter, implying a great potential for biorefinery as a feedstock. The chemical oxygen demand (COD) of cattle manure is about 0.9 g/g of dry matter or about 1 g/g of VS. This value is close to the theoretical COD/VS ratio of carbohydrates (1.07–1.18), which is lower than those of proteins (1.45) and lipids (>2, typically 2.9) [15]. This finding is consistent with the high fiber content (around 50%) and relatively low protein content (<20%). In addition, the biological oxygen demand (BOD) of cattle manure is about 20% COD, which indicates relatively low digestibility and is supported by high cellulose and lignin content that are known to be recalcitrant.

Table 1. Composition of cattle manure reported in the literature.

	Parameters	Dairy Manure	Beef Manure	Refs.
Basic Characteristics	Total Solids (%)	12.69–20.95	16.30	[16–23]
	Volatile Solids (%TS)	76.70–86.33	89.39	[18–22,24,25]
	COD (mg/kg DM)	910,112	939,394	[24]
	BOD (mg/kg DM)	146,067	212,121	[24]
	pH	6.8–7.3	n/a	[18,20]
Molecular Components	Fiber (%TS)	52.6	51.5	[17]
	Cellulose (%TS)	14.4–27.4	21.9	[17,18,20–23,26–29]
	Hemicellulose (%TS)	12.2–19.0	17.4	[17,20,22,26–29]
	Lignin (%TS)	8.1–16.0	12.2	[17,18,22,23,26,29,30]
	Crude Protein (%TS)	8.3–18.1	12.1	[17,31]
Macronutrients	Total Carbon (%TS)	35.05–45.37	43.83	[17,20–22,26]
	Total Nitrogen (%TS)	2.01–2.41	n/a	[21,22,27]
	Total-N (mg/kg DM)	30,300	19,392	[17]
	Organic-N (mg/kg DM)	29,571	n/a	[32]
	C/N	12.96–22.37	n/a	[18,20,22,23,27,29–31]
	NH ₄ -N (mg/kg DM)	6071	n/a	[32]
	NO ₃ -N (mg/kg DM)	71	n/a	[32]
	Phosphorus (mg/kg DM)	4800	4200	[17]
	Potassium (mg/kg DM)	28,600	14,386	[17]
	Sulfur (mg/kg DM)	3100	2499	[17]
	Calcium (mg/kg DM)	12,000	10,596	[17]
	Magnesium (mg/kg DM)	5500	2998	[17]
	Sodium (mg/DM)	4700	2500	[17]
Micronutrients	Copper (mg/kg DM)	30	1.4	[17]
	Zinc (mg/kg DM)	320	42	[17]
	Iron (mg/kg DM)	173–300	590	[17,20]
	Aluminum (mg/kg DM)	140	170	[17]
	Cobalt (mg/kg DM)	0.89	1.20	[17]
	Chromium (mg/kg DM)	2.1	2.1	[17]
	Manganese (mg/kg DM)	510	600	[17]
	Molybdenum (mg/kg DM)	2.6	2.4	[17]
	Nickel (mg/kg DM)	10	10	[17]
	Vanadium (mg/kg DM)	4.7	5.6	[17]
	Arsenic (mg/kg DM)	4.83	1.53	[10]

n/a: not available; REF: reference; COD: chemical oxygen demand; BOD: biological oxygen demand; DM: dry matter; note: cadmium and lead are below the determination limit.

Carbon is the most abundant element in cattle manure followed by nitrogen and other macronutrients, such as potassium, calcium, phosphorus, magnesium, sodium and sulfur, and micronutrients (Table 1). Most of the nitrogen in cattle manure is in the form of organic-N with about 20% NH₄-N and a small fraction of NO₃-N. The macro- and microelements in manure are crucial for both its biological and chemical conversion into value-added chem-

icals. In biological processes, elements like nitrogen, phosphorus, magnesium, calcium, and certain trace elements are essential for microbial metabolism. However, in chemical processes, some elements can adversely affect the conversion yield of the final products. For instance, a high nitrogen content can trigger the Maillard side reaction during the acid hydrolysis of manure fiber.

3. Fractionation of Cattle Manure or Cattle Manure Digestate

3.1. Fiber Recovery

Cattle manure fiber is an abundant and novel natural polymer with significant potential as a raw material for various industrial applications, such as paper production and bio-composites [33].

Vishnuvarthanan et al. [34] produced paperboard by combining cow manure and banana fibers through chemical pulping. The results demonstrated that the addition of cow manure significantly enhanced the paperboard's mechanical properties. The water absorbency increased, while the contact angle decreased. Despite a reduction in barrier properties, the material shows promise as an alternative barrier for packaging applications. Additionally, the paperboard exhibited strong antimicrobial activity against both *S. aureus* and *E. coli*, suggesting its potential for use in food packaging [34].

Composites are commonly used in the construction of buildings, bridges, and various structures, including boat hulls, swimming pool panels, racing car bodies, shower stalls, bathtubs, and storage tanks, as well as imitation granite and cultured marble sinks and countertops. The direct use of whole cow manure (including solids and undigested fibers) in composites may lead to variations in the material's properties due to the high solid content. Therefore, it is essential to conduct research that focuses on separating the fibers from the manure before using them in composite materials. Alkaline and hot water treatments are effective for removing lignin from the separated fibers. Polyethylene or biodegradable plastics can be suitable matrix resins when combined with these dung fibers [33,35–40].

3.2. Protein Recovery

Cattle manure contains a significant amount of protein, which is often underutilized and susceptible to runoff, leading to eutrophication. Although few studies have explored protein recovery from cattle manure, the results so far are promising. Vanotti and Szogi [41] developed a system and method for separating protein from raw manure using two steps of solid-liquid separation with acidic and basic treatments [41]. The basic treatment uses a high concentration of alkali chemicals (~1 M), and the alkaline needs to be separated from the protein and recycled to reduce the operational cost. Tasaki et al. [42] reported a “chemical-free” approach using the thermal hydrolysis process (THP) for protein recovery from manure solids that were separated by a screw separator from the cattle manure digestate after AD. The method allows precise control over the molecular weight distribution of the extracted protein hydrolysate. The nutritional value of the protein hydrolysate was assessed by analyzing its amino acid composition and molecular weight distribution through amino acid analysis, SDS-PAGE, and MALDI-TOF mass spectrometry. The amino acid (AA) composition of the protein hydrolysate was found to contain 2.5 times more essential AAs and 2.3 times more nitrogen content than soybean meal on a dry matter basis. Further studies on animal testing are suggested to confirm its nutritional value. Interestingly, the antioxidant activity of the protein hydrolysate, which was evaluated using the Oxygen Radical Absorbance Capacity Assay, was found to be comparable to that of vitamin E in terms of its antioxidant capacity against peroxyl radicals. Moreover, the antioxidant activity of the protein hydrolysate was seven times stronger than that of vitamin E in terms of its inhibition of hydroxyl radicals [43].

4. Platform Chemical Production from Cattle Manure

4.1. Volatile Fatty Acids

VFAs are short-chain aliphatic monocarboxylate compounds with one to seven carbon atoms, existing as either straight or branched chains, and include acids such as formic, acetic, propionic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, hexanoic, and heptanoic [44,45]. VFAs have gained attention in biorefinery due to the increasing demand for their broad applications. In brief, approximately 1.5 million metric tons of acetic acid were produced globally in 2022, and this number is expected to increase to 2.8 million metric tons by 2030 [46]. Similarly, the demands for butyric acid and propionic acid are also increasing due to their application in various industrial sectors, including biopolymers, bioplastics, animal feed, food, and pharmaceuticals [47]. Generally, VFAs are produced during acidification or fermentation, which follows the hydrolysis stage. The hydrolysis of cattle manure depends on its inherent characteristics, particularly cellulose, hemicellulose, and lignin, as well as other available soluble organic matter, such as long-chain fatty acids (LCFAs) and proteins. Various pretreatment approaches have been suggested to improve enzyme and microbial accessibility to these structural carbohydrates in cattle manure.

VFAs from the acidogenic fermentation of cattle manure can be distilled through solvent extraction, membrane separation, and pervaporation to obtain purified, high-value propionic and butyric acids, which are valued at 2.0 to 2.2 times the price of acetic acid [48–52]. In addition, mixed VFAs or VFA-containing digestate could further be valorized by different methods, including polymerization, chain elongation, esterification, algae cultivation, and lipid production with oleaginous microorganisms (microalgae, bacteria, fungi) [47,53].

Various metabolic pathways involved in the degradation of cattle manure by mixed cultures can affect VFA production during AD, with temperature and pH playing key roles. The biogas production of AD is usually inhibited when $\text{pH} < 6.5$ due to the arrestment of sophisticated methanogenic archaea, which allows VFA accumulation [18,54]. On the other hand, dark fermentation (hydrolysis–acetogenesis) primarily aims at producing biohydrogen or biohydrothane, with VFAs also generated as byproducts. However, excessive organic acid accumulation can inhibit biohydrogen production, particularly when the pH falls below 5.0 [55]. A two-stage AD process was developed, where the first-stage reactor operates under hydrolysis–acetogenesis conditions for VFA production, with the pH controlled between 5 and 6 and a hydraulic retention time (HRT) of 3 to 5 days [56]. A total VFA of 5–8 gHAc/L could be achieved in the first-stage reactor [19,20]. Without pH control, a sole substrate raw cattle manure could produce VFAs of 6.91 g-HAc/L after four days of acidification [19]. During the co-digestion of sweet sorghum stalks, cheese whey, and liquid cow manure, the optimal pH for both biohydrogen and VFA production was observed to be 5.5–6.0, with acetate and butyrate being the predominant VFAs (Figure 2 and Table 2) [57].

Thermophilic (55 °C) or hyperthermophilic (>60 °C) conditions can be applied in the first reactor for temperature-phased anaerobic digestion (TPAD) [30,58]. Hyperthermophiles from *Clostridia*, *Thermotogales*, and *Caldicellulosiruptor* are capable of degrading biomass complex carbohydrates to lactate, acetate, and hydrogen [21,59]. *Caldicellulosiruptor bescii* has been isolated for lignocellulosic biomass degradation with an acetic acid concentration of 5450 mg/L and a lactic acid concentration of 990 mg/L due to the availability of cellulase–cellulase multienzymes (Endoglucanase A or Cellulase A, CelA) [26,60]. VFAs produced during the thermophilic AD of cattle manure increased by 31.3% compared to mesophilic AD, probably due to a shift in microbial community where *Clostridia*, *Halanaerobiales*, and hydrogenotrophic methanogen (i.e., *Methanobacterium*) became predominant [61].

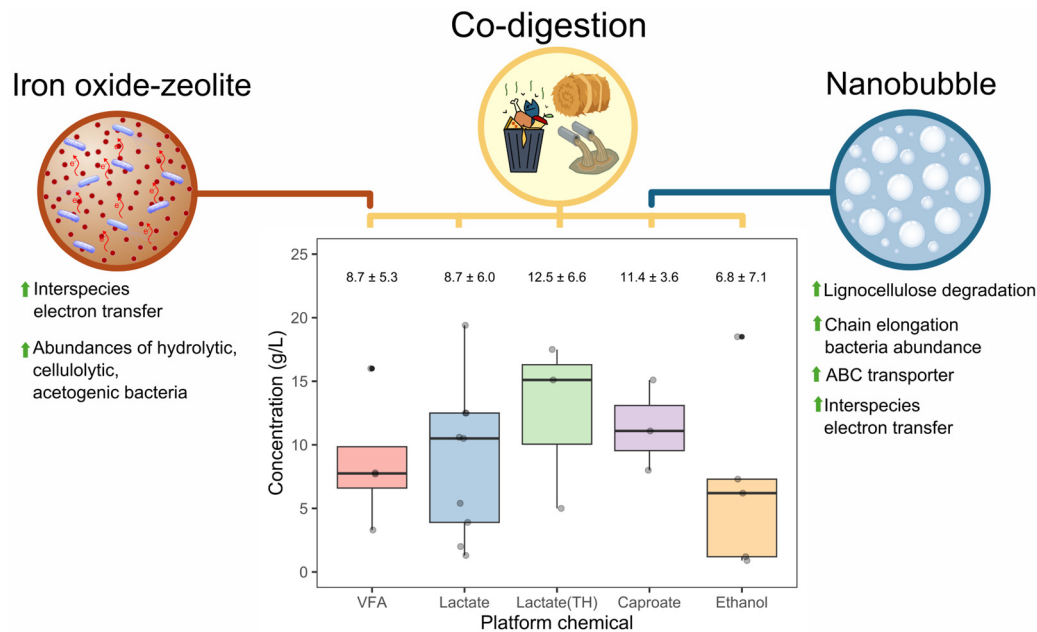


Figure 2. Platform chemical concentrations achieved by promising technologies. The data at the top is presented as mean ± SD. Gray dots represent the original values, and black dots indicate the outlines.

A catalyst addition, such as iron oxide–zeolite, could also enhance VFA production from cattle manure. The addition of 5% (*w/w*) iron oxide–zeolite to the co-digestion of cattle manure and pretreated rice straw (1:1.7 VS based) significantly improved lignocellulosic hydrolysis and acetogenesis, as indicated by a 21.5–27.3% increase in sCOD concentration, likely due to the inter-electron transfer (IET) facilitated by the conductive iron oxides [20]. In contrast, zerovalent iron has no effect on VFA concentration and inhibits biogas production in the AD of cattle manure and lignocellulosic biomass [22].

Cattle manure functions as both a substrate and an inoculum due to its rich microbial diversity, including over 60 bacterial species and 100 species of protozoa and yeast, such as *Citrobacter koseri*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Kluyvera* spp., *Morganella morganii* (lignolytic bacteria), *Pasteurella* spp., *Providencia alcaligenes*, *Providencia stuartii*, and *Pseudomonas* spp. [62]. Cattle manure contains cellulolytic microorganisms such as *Ruminofilibacter*, *Acetivibrio*, *Pseudomonas* spp., *Klebsiella pneumoniae*, and *Bathyarchaeota* (cellulolytic archaea) [27,62], as well as acetate producers *Prevotella*, *Clostridium*, and *Terrisporobacter* [63]. Additionally, cattle manure digestate also harbors cellulose and hemicellulose degrading bacteria (*Clostridiales* and *Prevotella*) and hydrogenotrophic archaea *Methanobrevibacter*, which play important roles in lignocellulosic biomass degradation (e.g., rice straw) [64]. Furthermore, bioaugmentation such as cow digestion solution (CD) and a strain consortium (SC) (*Clostridium fungisolvans*, *Clostridium sensu stricto*, and *Clostridium beijerinckii*) could improve 66.7% of VFA production [65]. Up to 16 g/L of VFAs can be produced when using a mixture of olive mill wastewater, cheese whey, and cattle manure (55:40:5, *v/v*), with cattle manure contributing even as a small portion of the substrate, likely serving as a microbial source [57,66].

Table 2. Platform and high-value chemicals from the fermentation of cattle manure.

Co-Substrate		Vol (L)	Temp (°C)	Loading	Duration (d)	pH	Concentration (g/L)				Refs.
Type	Co-Substrate Ratio						VFA	Lactate	Ethanol	Caproate	
Mono-digestion		0.03	75	50 gVS/L	45	5.8	5.4	1.0			[26]
Mono-digestion		0.70	37	60% <i>w/w</i>	4	6.3	7.7				[19]
Mono-digestion			50	80 g/L	2			15.1			1 [67]
Mono-digestion		0.18	20–23			4.0		5.4	1.2		[68]
Mono-digestion		20.0	55	15–25 g/L·d	HRT = 40–67	5.5		5.0			2 [69]
Mono-digestion		0.40	37		6	6.0		2			[63]
Mono-digestion		0.30	30		3	10.0		1.3			[70]
Mono-digestion		0.02	30	10% <i>w/v</i>	24 h	4.8			18.5		3 [28]
Mono-digestion		0.05	30	10% <i>w/v</i>	6 h	5.0			7.3		4 [71]
CM:RS	1:1.7 VS	5.50	35	8 gVS/L·d	HRT = 12	6.8	7.8				5 [20]
CM:FW	40:60 VS	0.40	35	50 g/L·d	HRT = 2	3.8		19.4	6.2		6 [72]
CM:FW	1:1 mass	0.70	35	8%TS	45	6.0	3.3				7 [18]
	91:0:9					3.6	28 *	24.2 *	16.6 *		
CM:WS:Glu	85:6:9	2.55	25		30	3.6	21 *	18.4 *	11.4 *		8 [73]
	80:12:8 mass					3.9	13 *	13.0 *	10.2 *		
SSS:CW:LCM	55:40:5 vol	0.90	37			5.5	16		0.9		9 [57]
			35			4.9		10.6			
CM:CSS	1:3 VS	0.90	50	4%TS	20	4.7		17.5			10 [31]
Olive mill WW:CW: LCM	55:40:5 vol	0.40	37	85% <i>v/v</i>	21	6.0		10.5			11 [66]
Leachate					46	7.0		3.9			[74]
					1.2	4.5		12.5			
SHS:WP:GB:water:CM	31:26:20:14:9 mass	5.00	40		1.1	5.0		12.5			[25]
Lactic acid	LA:VFA = 3 mass	0.40	30	71.5 g/L	18	5.5				8.0	12 [23]
Ethanol	ED: EA = 3 mass	0.12	30	4.49%VS	17	5.9				15.1 (NBW)	13 [75]
										11.09	

Note: * %VS_{added}, CM: cattle manure; CW: cheese whey; CSS: corn straw silage; ED: electron donor; EA: electron acceptor; FW: food waste; GB: green bean; Glu: glucose; LCM: liquid cow manure; RS: rice straw; SHS: slaughterhouse sludge; SSS: sweet sorghum stalk; Vol: Working volume; WP: whey permeate; WS: wheat straw; WW: wastewater. Remarks: ¹ Pretreatment: Enzyme 212.5 IU/gCM_{DM}. ² Semi-continuous. ³ Pretreatment: NaOH 0.2 g/g CM, 120 °C 30 min, pre, and post-dry 45 °C, Cellulase SN-1 20 FPU/g-dried substrates. ⁴ Pretreatment: 2.5% H₂SO₄ at 121 °C 30 min, 20 FPU/gDM. ⁵ Semi-continuous. Iron oxide–zeolite improved VFA production under ambient temperature. ⁶ Batch (1.5 d), Semi-continuous (15 d). Hydrogen yield has a moderate negative correlation with lactate and a moderate positive correlation with propionate and valerate. Zero-hydrogen heterolactic fermentation becomes the dominant metabolic pathway when pH < 4. ⁷ Controlled pH. Uncontrolled pH (5.5) also achieved the same VFAs at the end of the experiment. ⁸ Neglectable amounts of lactate and ethanol produced with CM: Starch = 87.7:7.0:5.3 mass. ⁹ Heat-treated anaerobic sludge inoculum improved bio-hydrogen production, lactate was 7 g/L after 6 days of fermentation. ¹⁰ Under thermophilic conditions, CSS increased the relative abundance of Lactobacillus for LA production. ¹¹ pH is controlled. ¹² Additive NBW: Air nanobubble water with 100 mL added. ¹³ Lactic acid = 29.5 g/L, Fermentation 7 d, Chain elongation 10 d, acetic acid = 4.05 g/L was produced during fermentation stage. Intrinsic chain elongation bacteria: *Clostridium*, *Eubacterium*, and *Caproiciproducens*.

4.2. Lactic Acid

Cattle manure contains key microbiomes for lactate production with three *Lactobacillus* strains, including *Lactobacillus gasseri*, *Lactobacillus reuteri*, and *Lactobacillus salivarius* [76]. Cattle manure alone with enzyme hydrolysis could produce lactic acid up to 15.1 g/L. Lactic acid was not consumed at pH 4.5 and 5, and its accumulation inhibited other metabolic pathways, indicating lactic fermentation [25]. Another study also showed the potential of VFAs and ethanol co-production. The co-digestion of cattle manure and food waste (40:60, VS based) can produce up to 19.4 g/L of lactic acid and 6.2 g/L of ethanol [72]. This finding is likely due to the dominance of zero-hydrogen heterolactic fermentation, driven by the rapid biodegradability and diverse carbohydrates and proteins in the food waste and lactic acid in the cattle manure [72,77].

4.3. Medium-Chain Carboxylic Acids

Caproic acid can be produced through a reverse beta-oxidation cycle during anaerobic fermentation when electron donors such as ethanol and lactic acid are added, with available short-chain carboxylic acids (SCCAs) acting as electron acceptors [75,78]. Due to its ability to produce SCCAs, cattle manure can generate approximately 10.0–11.0 g/L of caproic acid when lactic acid or ethanol is added in an electron donor to electron acceptor ratio of 3:1 (*w/w*) [23,75]. The same research group also found that nanobubble aeration enhances caproic acid production by up to 55.0% (15.1 g/L), compared to the control, as well as increases the levels of heptanoic and caprylic acids to 1.12 and 0.22 g/L, respectively. This improvement is attributed to its ability to break down the cellulose matrix and further decompose recalcitrant material in cattle manure. Additionally, key chain elongation and electron transfer bacteria are enriched (*Bacillus* and *Caproiciproducens*, *Syntrophomonas*, respectively) [23]. Yin et al. [75] also support the idea that intrinsic chain elongation bacteria, such as *Clostridium*, *Eubacterium*, and *Caproiciproducens*, play a crucial role in caproic acid production in cattle manure wastewater, although substantial amounts of heptanoic and caprylic acids were not observed [75].

4.4. Ethanol

Bioethanol is not only profitable as a fuel in gasoline-ethanol blends but also serves as a platform chemical for producing high-value products, such as ethylene oxide, 1-butanol, acrylic acid, and acetaldehyde [79]. Different yeast strains have been used to produce ethanol from cattle manure hydrolysate. Alkali pretreatment was proposed based on cellulose and hemicellulose (35.4 and 15.5%DM, respectively) availability in hydrolysate [28], probably due to the fact that alkaline pretreatment effectively solubilizes hemicellulose and removes lignin while preserving cellulose. Unlike acid pretreatment, which can lead to cellulose hydrolysis, alkaline pretreatment reduces amorphous components, exposing more crystalline cellulose by disrupting the cellulose–hemicellulose–lignin network [80]. Yan et al. [71] obtained 18.9 g/L of ethanol using *S. cerevisiae* LF1 with 10%*w/v* alkali-pretreated cattle manure after cellulose SN-1 hydrolysis. On the other hand, cattle manure acid hydrolysate (10%*w/v*) only produced 7.3 g/L of ethanol from batch fermentation using Thermosacc® dry *S. cerevisiae* [71]. In advance, alcohol could be produced up to 25.65 g/L in fed-batch enzymatic hydrolysis of 10%*w/v* alkali pretreated cattle manure with an initial hydrolysis of 20 FPU/g cellulase SN-1 and an additional 10%*w/v* pretreated biomass with *S. cerevisiae* LF1 [28]. Indeed, ethanol fermentation depends on several parameters; however, data suggest that alkali pretreatment is comparatively more efficient for the ethanol fermentation of cattle manure. More advanced methods could be employed, but their use also depends on economic considerations.

5. Cattle Manure Digestate Biorefinery for Platform Chemical Production

The downstream management of cattle manure AD digestate has contributed to environmental impacts and pollution in many aspects. For example, the spread of digestate could affect aquatic acidification and eutrophication [81]. The systematic review of life

cycle analyses pointed out that the land application of cattle manure digestate may lower GHG emissions more than fresh manure since AD reduces the organic matter and ammonia volatilization [82]. Cattle manure fertilizer utilization yields a three-fold increase in marginal environmental benefits compared to direct land application [83]. However, further treatment, including dewatering, wastewater treatment, and composting, could reduce up to 50% of acidification potential but result in higher global warming potential [84]. While the direct application of digestate improves soil nitrogen availability, high VFA levels can pose a risk of inhibiting the conversion of ammonia into nitrate, a plant-ready nitrogen source [85,86]. Additionally, during storage, ammonia and methane gases are still emitted, contributing to GHG emissions due to the continuation of degradation [82]. Therefore, digestate storage is crucial for pollution control. Another study has shown that, with closed digestate storage and renewable energy-powered electricity, the co-digestion of cow manure with cow feed waste, sewage sludge, and returned dairy products has the best environmental performance in global warming, eutrophication, and acidification, compared to mono-digestion and co-digestion with maize straw; it was the most economically viable, with a 14% internal rate of return and a 15% return on investment [87]. Even though the perspectives of digestate valorization from a life cycle assessment point-of-view have not been explored, the organic matter could still be utilized for secondary biorefinery.

Residue organic matter and VFAs in cattle manure digestate can serve as a valuable carbon source for single-cell proteins (SCPs) and oleaginous microorganisms. Several studies have demonstrated the production of SCPs and lipids from a sole VFA mixture. For example, an SCP yield of 0.76 g/L was produced from digestate forward osmosis concentrate containing approximately 6.0 g/L of acetate and 0.8 gN/L of NH_4 [88]. Oleaginous microorganisms can also utilize VFAs for lipid production, with yields ranging from 0.08 to 0.14 g_{lipid}/g_{VFA} [89,90]. Microalgae (*Auxenochlorella protothecoides* and *Chlorella sorokiniana*) have been reported to produce 20–40%w/w_{DCW} (w/w-dry cell weight) of lipids from 5 to 10 g/L of mixed VFAs with C/N = 20 using yeast extract as a nitrogen source [91]. Additionally, *Cryptococcus curvatus* produced 28% and 41%w/w_{DCW} of lipids from waste office paper and waste newspaper digestates, respectively. *Yarrowia lipolytica* has been shown to produce lipids up to 10.1 g/L (27%w/w_{DCW}) when higher alkalinity (pH = 8) alleviates the very high acetic acid concentration of 70 g/L [90]. Therefore, given its high alkalinity and VFA availability, cattle manure digestate should be a promising candidate for oleaginous microorganism cultivation.

6. Process Integration

High-value products can be recovered from cattle manure through biological conversions (such as AD, dark fermentation, and ethanol fermentation) by integrating with various post-processing techniques (Figure 3). Depending on the concentration of substances, which is influenced by the conditions of the main fermentation processes, VFAs, lactic acid, MCCAs, and ethanol can be purified directly from cattle manure digestate using pervaporation, distillation, or solvent extraction. Alternatively, a dewatering unit can be employed to extract VFA-rich liquid digestate for auxiliary secondary bioprocess, including algae production [92,93], single-cell protein cultivation [88], and oleaginous microorganism cultivation [94–96]; e.g., agro-zootechnical biomethane plants in which a raceway pond and harvesting system were employed in the AD plant [97]. Algal and single-cell proteins can be used to produce high-nutrient products, such as livestock and animal feed. Oleaginous microorganisms (e.g., yeast and algae) can utilize VFAs as a sole carbon source to produce lipids for applications like bio-oil, biofuels, and lubricants.

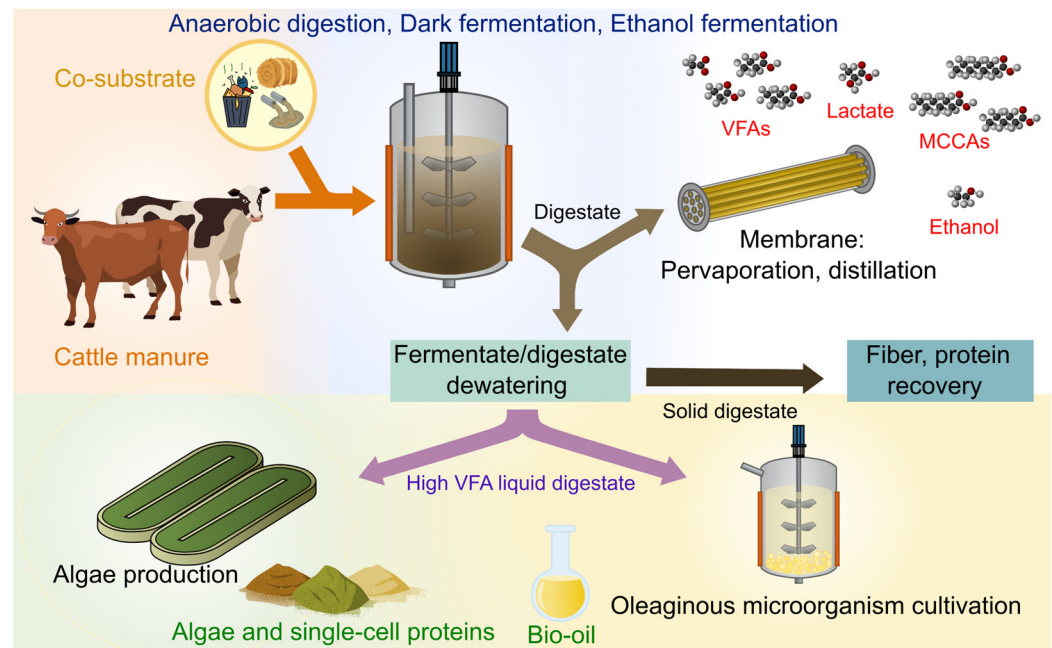


Figure 3. Process integration framework of cattle manure biorefinery for platform chemicals.

7. Feasibility and Perspectives

The feasibility and scalability of cattle manure biorefinery have been analyzed in several aspects, including pretreatment, co-digestion, and two-stage configuration. Even though the integrated process abovementioned has not been incorporated, the pretreatment of cattle manure for cellulose accessibility, as well as the co-digestion for higher platform chemical yields, are crucial, encompassing economical, sustainable, and environmental factors. Lactate produced from cow manure could be sold at € 0.945/kg, which is comparable to commercial starch-base lactate with a positive return on investment (16.7%) and break-even point (21% the total capacity) [3]. From a pretreatment perspective, solar thermal pretreatment of cattle manure (40–60 °C, 20 h) has shown a 17.2% higher revenue with 3.9-fold methane production compared to those without pretreatment [98]. Similarly, thermal-acid pretreatment with 0.5% of HCl at 37 °C for 12 h is still profitable (USD 0.94 MM/y), even less compared to a single-stage AD (USD 1.03 MM/y), considering biogas alone [99].

Co-digestion could result in a higher net present value and lower total life cycle emission due to electricity generation and a synthetic fertilizer replacement, compared to cattle manure mono-digestion [100]. For example, the co-digestion of cattle manure, biomass, and glycerine reduced operating costs by 32% and increased the return on investment by 27% [101]. Even though co-digestion at a high organic loading rate is economically favorable in dairy farms, it might pose risks of remaining COD and methane emissions downstream [30]. Co-digestion with bioenergy crops such as sorghum and miscanthus could reduce the impact of climate change by 90–105% and 60–80%, respectively [102]. The integration approach of pretreatment and co-digestion may even yield higher energy output. Microwave thermohydrolysis (150 °C, 15 min) of cattle manure and *Sida hermaphrodita*, contributed to higher net energy output at 4.20 Wh/g VS, compared to the control at 3.75 Wh/g VS, suggesting MW pretreatment with less than 2.65 kJ/g VS of energy with thickened and/or dewatered co-substrate to achieve a positive energy balance [103]. Additionally, in the full-scale study (20.9 m³), hydrodynamic cavitation pretreatment of cattle manure and wheat straw co-digestion produced 8.9% higher net energy output (61.05 kWh/d) compared to the non-pretreatment [104]. Another full-scale two-stage study, incorporating a food waste hydrolysis tank fed into a cattle manure digester, achieved specific methane yields of up to 600 NL/kg VS at OLR = 3.0–3.5 kgVS/L·d with a predomination of lactate

and an acetate fermenter in the hydrolysis tank and hydrogenotrophic methanogens in the main digester [105].

Although individual cattle manure biorefinery technology for added-value products has been evaluated, the effects of micropollutants in platform chemical production remain unexplored. For example, the effects and fates of antibiotics used in cattle industries on bioprocess efficiency, such as cefazolin, are necessary [19]. Advanced bioprocesses such as microbial electrolysis [106], the membrane aerated bioreactor (MABR), the membrane anaerobic bioreactor (MAnBR), and the microbial fuel cell (MFC) offer promising avenues for exploration. Essentially, genetic modification, such as RNA interference, clustered regularly interspaced short palindromic repeats (CRISPRs), and transcription activator-like effector nucleases (TALENs), might improve the degradation of refractory lignocellulosic fibers in cattle manure, minimizing the pretreatment step, as well as maintain the integrity of platform chemical production [107,108]. Novel separation techniques, such as bicontinuous polyelectrolyte complex (PEC)-layered nanofiltration membranes [109], are also necessary to enable efficient recovery with economic viability. Ultimately, the economic feasibility and life cycle assessment with different scenarios based on the spatiotemporal availability should be further exploited.

Table 3 below further indicates the potential of producing value-added products from cattle manure worldwide. Globally, 3.4 million dry tons of cattle manure could provide 510 million tons of crude protein and 850 million tons of cellulose, or 2958 million tons of VS, which are potential feedstocks for the production of protein (179–306 million tons), paper/composites (383–425 million tons), ethanol (162 million tons), or PHA (354 million tons), PLA (621 million tons), and aviation fuels (446 million tons) (Table 3). The potential of these products from cattle manure is higher than or comparable to their total global demand. It should be noted that the potential of VS overlaps with that of crude protein and cellulose, meaning cattle manure cannot meet the demand for all potential products at the same time. As a result, it remains crucial to explore other biomass resources, such as energy crops, agricultural residues, forestry waste, and municipal waste, to improve sustainability through biorefinery processes.

Table 3. Global potential of value-added products from cattle manure.

Cattle Manure Production (Million Dry Metric Tons)	Potential Feedstocks from Cattle Manure		Potential Products from Cattle Manure			
	Type	Amount (Million Metric Tons) ²	Type	Conversion Efficiency (%)	Potential Production (Million Metric Tons) ³	Total Demand (Million Metric Tons)
3400 ¹	Crude Protein	510	Protein	35–60 ⁴	179–306	97 ⁵
	Cellulose	850	Paper Composite	45–50 ⁶	383–425	406 ⁷ 10 ⁸
			Ethanol	19 ⁹	162	131 ¹⁰
	VS	2958	PHA PLA	12 ¹¹ 21 ¹²	354 621	460 ¹³
			Aviation Fuels	15 ¹⁴	446	300 ¹⁵

¹: data from [3]; ²: calculated based on composition values in Table 1; ³: calculated based on amount of potential feedstock and conversion efficiency; ⁴: data from [42]; ⁵: data from [110]; ⁶: data from [111]; ⁷: data from [33]; ⁸: data [112]; ⁹: data from [28]; ¹⁰: data from [113]; ¹¹: VS to PHA conversion efficiency calculated with VS to VFA yield of 0.26 (g/g) and VFA to PHA yield of 0.46 (g/g) (data from [114] and [115], respectively); ¹²: VS to PLA conversion efficiency calculated with VS content, dry cow manure to lactic acid yield of 0.33% (g/g), and lactic acid to PLA yield of 0.55 (g/g) (data from Table 1, [3] and [116], respectively); ¹³: data from [117]; ¹⁴: VS to Aviation fuels conversion efficiency calculated with VS to VFA yield of 0.26 (g/g) and VFA to Aviation Fuel yield of 0.58 (data from [114] and [118], respectively); ¹⁵: data from [119].

8. Conclusions

The high organic content and nutrient profile of cattle manure offer significant potential for biorefinery applications. While cattle manure fibers show promise in enhancing the properties of paperboard and composites, proper processing and treatment, such as lignin removal, are crucial for maximizing their potential and ensuring consistency in material performance. Recovering protein from cattle manure or its digestate addresses environmental concerns and provides valuable, nutritionally rich, and functional materials. Advances in recovery methods, particularly the chemical-free thermal hydrolysis approach, highlight promising avenues for the sustainable utilization of manure resources. Optimizing hydrolysis, pretreatment, and processing conditions in cattle manure can significantly enhance the production of high-value platform chemicals such as VFAs, lactic acid, caproic acid, and ethanol. Excess VFAs in cattle manure digestate offer a valuable carbon source for producing single-cell proteins and lipids, especially with the optimization of conditions. Economic and methodological considerations are essential in selecting the most effective approaches for maximizing yields. Future research on system modification and genetic engineering is essential to advance the State-of-the-Art in cattle manure biorefinery.

Author Contributions: Conceptualization, W.C. and A.S.; investigation, W.C., J.G. and A.P.T.; writing—original draft preparation, W.C., J.G. and A.P.T.; writing—review and editing, W.C., A.P.T. and A.S.; visualization, W.C.; supervision, A.P.T. and A.S.; project administration, A.S.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) through Award 2023-67019-39731.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

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