

# Journal Pre-proof

Biorefinery development in livestock production systems: Applications, challenges, and future research directions

Giovanni Ferrari, Giorgio Provolo, Stefania Pindozi, Francesco Marinello, Andrea Pezzuolo



PII: S0959-6526(24)00305-6

DOI: <https://doi.org/10.1016/j.jclepro.2024.140858>

Reference: JCLP 140858

To appear in: *Journal of Cleaner Production*

Received Date: 3 August 2023

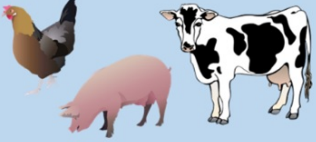
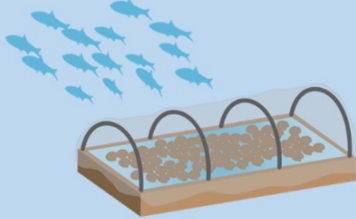


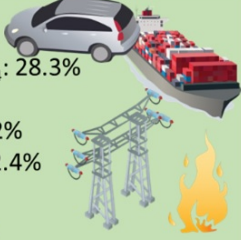



Revised Date: 28 December 2023

Accepted Date: 19 January 2024

Please cite this article as: Ferrari G, Provolo G, Pindozi S, Marinello F, Pezzuolo A, Biorefinery development in livestock production systems: Applications, challenges, and future research directions, *Journal of Cleaner Production* (2024), doi: <https://doi.org/10.1016/j.jclepro.2024.140858>.

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Livestock effluents and by products	Biorefinery treatment	Bioproducts
<p>Livestock farming: Cattle, pig, poultry</p>  <p>Fish products: Aquaculture, fish industry</p>  <p>Other: Sheep, horse, insects.</p>	<p>On-farm treatments (33.5%): Mainly anaerobic digestion, biogas upgrading, animal feed preparation</p>  <p>Industrial treatments (66.5%): Mainly biological and chemical treatments, microalgae cultivation, water purification</p> 	<p>Bioenergy:</p> <ul style="list-style-type: none"> <li>Biogas and CH<sub>4</sub>: 28.3%</li> <li>Biofuel: 7.5%</li> <li>Bioethanol: 9.2%</li> <li>Biohydrogen: 2.4%</li> </ul>  <p>Purified water: 3.4%</p>  <p>Animal feed: 28.7%</p> <p>Fertilizer: 5.8%</p>  <p>Manufacture: 4.1%</p> <p>Pharma industry: 2.7%</p> <p>Other: 7.9%</p> 

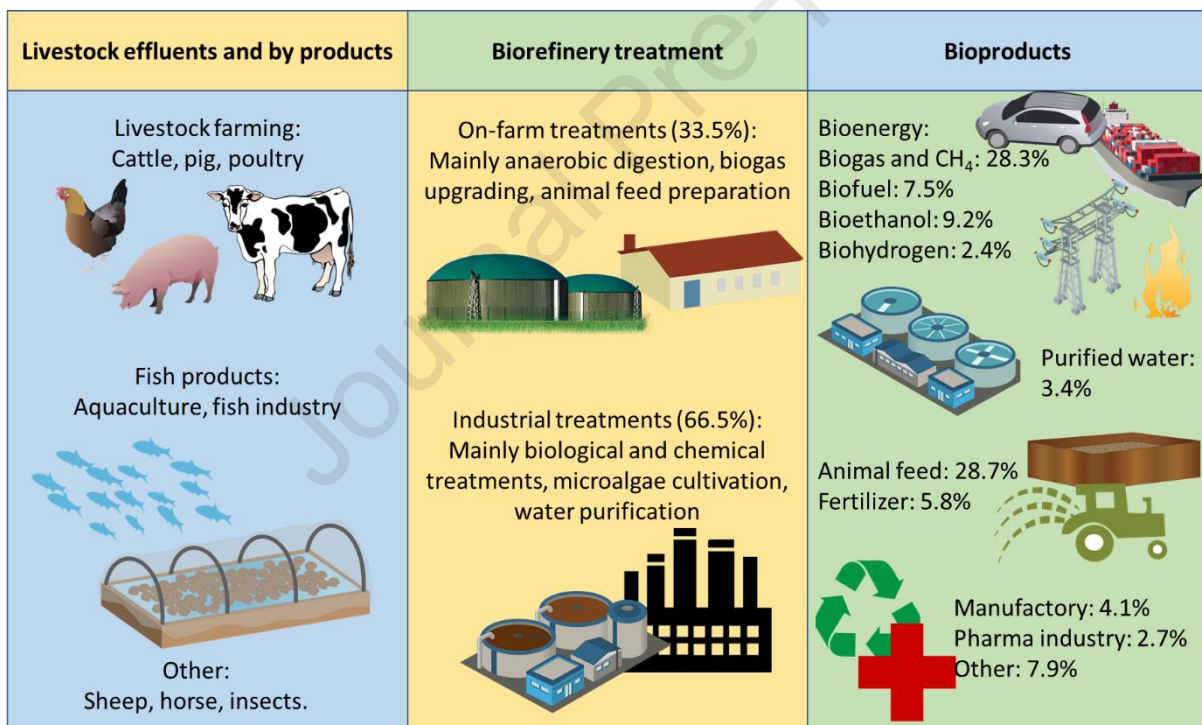


31 growing interest in insect cultivation. In the coming years, one of the most significant challenges will  
 32 be the successful transfer of technologies and processes from experimental research to the applied  
 33 industry. To do this, it will be necessary to reduce costs, exploit economies of scale, improve process  
 34 management, and develop synergies between different industrial sectors to implement smart circular  
 35 economy systems. Overall, this review aims to clarify the hypothesis driving research in this area and  
 36 emphasizes the tangible applications of findings within the broader context of sustainable resource  
 37 management.

38

39 **Keywords:** Livestock manure, circular economy, nutrient recovery, bioproducts, bioenergy.

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41

42

## 43 1 Introduction

44 The social importance of livestock farming goes far beyond job creation: many European cultural  
 45 landscapes and traditions have developed alongside livestock production (Herrero et al., 2013). It is  
 46 an essential part of the economy and culture of many regions, including many marginal areas in rural

47 areas of Arava (Israel), Murcia (Spain), La Vallée de la Drôme (France), Salzburg region (Austria)  
48 and Tuscany (Italy) (de Roest et al., 2018). The importance of this sector for the economy and the  
49 environmental, industrial and energy policies of the European Union (EU) and its member states is  
50 evidenced by the high number of animal units achieved (142 million pigs, 76 million bovine animals,  
51 60 million sheep and 11 million goats in December 2021) (Scarlat et al., 2018b) (Eurostat, 2021).

52 From a circular bioeconomy perspective, livestock farming has many other important roles: *i*)  
53 contributing to more efficient agriculture through the exploitation and valorisation of byproducts in  
54 the food chain, recycling inedible biomass and deriving new sources of protein for animals (Farias et  
55 al., 2020); regulating ecological cycles, closing nutrient cycles, and increasing soil fertility and carbon  
56 sequestration through recycling and utilisation of manure as a bioresource in combination with fodder  
57 (Chiumenti et al., 2019; Hilimire, 2011); *ii*) providing feedstock for renewable energy production and  
58 thus contributing to the transition to renewable energy and byproduct production for the industrial  
59 sector (e.g., for animal feed, cosmetics, textiles, pharmaceutical industry) (Economics and Library,  
60 2010; Ferrari et al., 2022); and *iii*) providing ecosystem services essential for the vitality of territories,  
61 rural employment, landscape conservation, biodiversity, and cultural heritage (Dumont et al., 2019;  
62 Rodríguez-Ortega et al., 2014). In addition, it is possible to use the effluent produced by farmed  
63 animals to produce biogas, biomethane, and electricity; thus it is possible to turn a waste into an  
64 alternative energy source (Scarlat et al., 2018a).

65 However, livestock farming also has negative impacts on the environment due to the consumption of  
66 limited resources (land, water, and energy) (Ferrari et al., 2021b) and the production of flows of  
67 nutrients, greenhouse gases, toxic substances, etc., which can affect biodiversity, human health, and  
68 ultimately the functionality of ecosystems on which communities depend for food production  
69 (Peyraud and MacLeod, 2020). Livestock farming contributes to climate change by emitting  
70 greenhouse gases, both directly (e.g., through enteric fermentation) and indirectly (e.g., through feed  
71 production activities and deforestation). According to FAO results, livestock activities were  
72 responsible for the emission of 8.1 Gt CO<sub>2</sub>eq in the world and 0.25 Gt CO<sub>2</sub>eq in Europe (10% of total

73 emissions in EU-28) in 2017 (Peyraud and MacLeod, 2020); these gases consist mostly of methane  
74 (50%), nitrous oxide (N<sub>2</sub>O) (24%) and carbon dioxide (CO<sub>2</sub>) (26%) (Steinfeld et al., 2007). Analysing  
75 by species, cattle are the most significant contributors (37.0% beef, 19.8% dairy cattle), followed by  
76 pigs (10.1%) and poultry (9.8%) (Peyraud and MacLeod, 2020). Moreover, the high numbers of  
77 animal units have often been associated with soil pollution due to the disposal of nitrogen in sewage  
78 (Ferrari et al., 2021a).

79 In recent years, the EU and its member states have issued various regulations, directives and laws  
80 concerning livestock farming and biomass management (Directive 2001/81/EC, 2001; European  
81 Commission, 1991). These regulations were studied by Velthof et al., 2015, who reviewed the  
82 nitrogen excretion factors applied to a number of animal categories in policy reports from different  
83 EU member states. This work has also been done by other authors over the years, the results were  
84 also very different from each other, this is because of the different type of breeding and environmental  
85 conditions (Bao et al., 2019). Additionally, Wieruszewski and Mydlarz (2022) discussed the  
86 information gathered on biomass energy to achieve EU energy targets. The regulatory system for  
87 biorefineries in Europe is extensive. In some cases, these are documents specifically dedicated to this  
88 topic; more often, they are included in more comprehensive measures concerning sustainable  
89 development and energy transition.

90 One of the earliest EU acts was the Council Directive 91/676/EEC concerning the protection of water  
91 against pollution caused by nitrates from agricultural sources: the “Nitrates Directive” (European  
92 Commission, 1991). The directive prescribes the determination of water bodies vulnerable to nitrate  
93 pollution and their water catchment areas. The directive states that the amount of nitrogen that may  
94 be introduced into soils in these areas may not exceed 170 kg/ha/year. The European legislation  
95 requires that alternative solutions for the treatment of livestock manure must be adopted to comply  
96 with these limits. These solutions do not exclude the use of manure as fertiliser but involve more  
97 elaboration that could be facilitated by energy production, as in the case of biorefineries. In fact, these  
98 processes also allow alternative products, such as bioproducts and bioenergy, to be obtained.



99 The International Energy Agency Bioenergy Task 42 provided the definition of biorefinery: “the  
100 sustainable processing of biomass into a spectrum of biobased products (food, feed, chemicals,  
101 materials) and bioenergy (biofuels, power and/or heat)” (International Energy Agency - Bioenergy  
102 Task 42, 2019). Using biomass as a raw material can provide a benefit by reducing the environmental  
103 impact and greenhouse gas emissions for producing bioproducts (Bajpai, 2013). Biorefineries can be  
104 classified according to four characteristics (Cherubini et al., 2009):

- 105 - Platform. These are the intermediate products between the raw materials and the final bioproducts.  
106 The most important are biogas, syngas, hydrogen, carbohydrates, lignin, and oils.
- 107 - Bioproducts. These are the final products and can be of two types: energy (electricity, heat) or  
108 materials (for different types of industry).
- 109 - Raw materials. They can be dedicated biomass (energy crops, forest products) or waste and  
110 byproducts (including livestock manure).
- 111 - Type of process used. They can be of different types, even in combination: thermal, chemical,  
112 mechanical, and biological.

113 In this research, the previous classification was used, indicating “Platform” with “bioproduct  
114 produced”, “Bioproducts” with “Destination of bioproduct”, and “Raw materials” with “Biomass  
115 used”.

116 Biorefineries contribute to a more sustainable industrial system by preserving resources and reducing  
117 greenhouse gas emissions (Rekleitis et al., 2020). Nevertheless, the production of biomaterials entails  
118 other types of environmental impacts: land use, water eutrophication, and high energy demand  
119 (Biswal et al., 2020). To assess these impacts, an essential tool is life cycle assessment (LCA), which  
120 evaluates the environmental impact of a product or process from raw material to end-of-life disposal  
121 (Jacquemin et al., 2012). A certain number of LCAs have been published to analyse the environmental  
122 impact of biorefineries in comparison with traditional production systems; in addition, many  
123 technoeconomic analyses have been published concerning the processes and biomasses involved.  
124 This large amount of published research has produced numerous results, necessitating the publication

125 of specific review articles on a particular treatment adopted, a specific biomass used or a certain  
126 bioproduct obtained. At this point, it is necessary to understand how the various topics, products and  
127 techniques integrate and how the authors decided to deal with them: technical articles, review articles,  
128 LCA. For this reason, a systematic review of the literature on this topic is necessary.

129 This paper proposes a systematic review of articles published over the past 11 years concerning  
130 biorefineries applied to byproducts and waste from livestock farming. A large set of articles has been  
131 examined in an attempt to extract the essential information on the applied biorefinery processes at  
132 different scales (laboratory, pilot and full scale), the most successful pretreatments used, and the  
133 possible biorefinery outputs. A special focus was devoted to reviews, LCAs and techno-economic  
134 assessments to better define the directions of scientific research, technical applications, and the  
135 environmental consequences of these processes. Through this holistic approach, this research aims to  
136 take a detailed look at the biomass used in refinery processes and, through the systematic analysis of  
137 these data, interpret the research trend over the years, provide key elements for understanding this  
138 phenomenon for political decision-makers and propose new routes for research.

139



## 140 2 Field of analysis and research methodology

141 The methodology applied in this research consists of three stages. First is the definition of the analysis  
142 field, with the fundamental concepts for the search. Second, the search string on Scopus was  
143 described, and articles were identified. Third, relevant information was extracted from the selected  
144 articles and their analysis and discussion.

145 This study analysed research on biorefineries applied to the livestock sector, with a particular interest  
146 in managing and valorising manure and wastewater. Based on the objectives of the research, two key  
147 concepts were established and were used to define the search string on the Scopus database (Fig. 1):  
148 (i) biorefinery, regarding the way biomass is managed, and (ii) animal and livestock, regarding the  
149 scope of application. The two concepts were converted into two sets of search terms for the articles.  
150 Concerning the first concept, the search focused on the works the relevant authors considered related  
151 to the biorefinery, defined as a series of organised processes for biomass valorisation. The string used  
152 for the research was (*biorefinery or biorefineries or biorefining or biorefiner\**) and (*fish or*  
153 *aquaculture or insect or goat or sheep or livestock or cattle or pigs or poultry or swine or cow or*  
154 *dairy or beef or manure or slurry*). This string was applied through the title-abstracts-keywords  
155 indexed by the Scopus database, as it collects most scientific publications. This also allows for the  
156 search to be refined using a series of filters, particularly articles from 2012 to the present, in English,  
157 and only articles, reviews and conference papers were selected. This choice made it possible to  
158 include many articles to establish a more complete framework of the topic. The downside of this  
159 choice was that on examining the articles individually, many (almost half) were found to be unrelated  
160 to the topic and therefore not usable; this was because, in the abstract, the words of the research string  
161 were randomly present, but the actual topic was different from the targeted research areas.

162 The search produced 578 articles published between 2012 and 2022. The articles were analysed  
163 individually and filtered to select only those relevant to the research. Among the articles that  
164 contained search terms, only those that applied biorefinery processes to livestock biomass or  
165 produced livestock-specific products with such processes, e.g., feed or supplements, were included.

166 The following exclusion criteria were used:

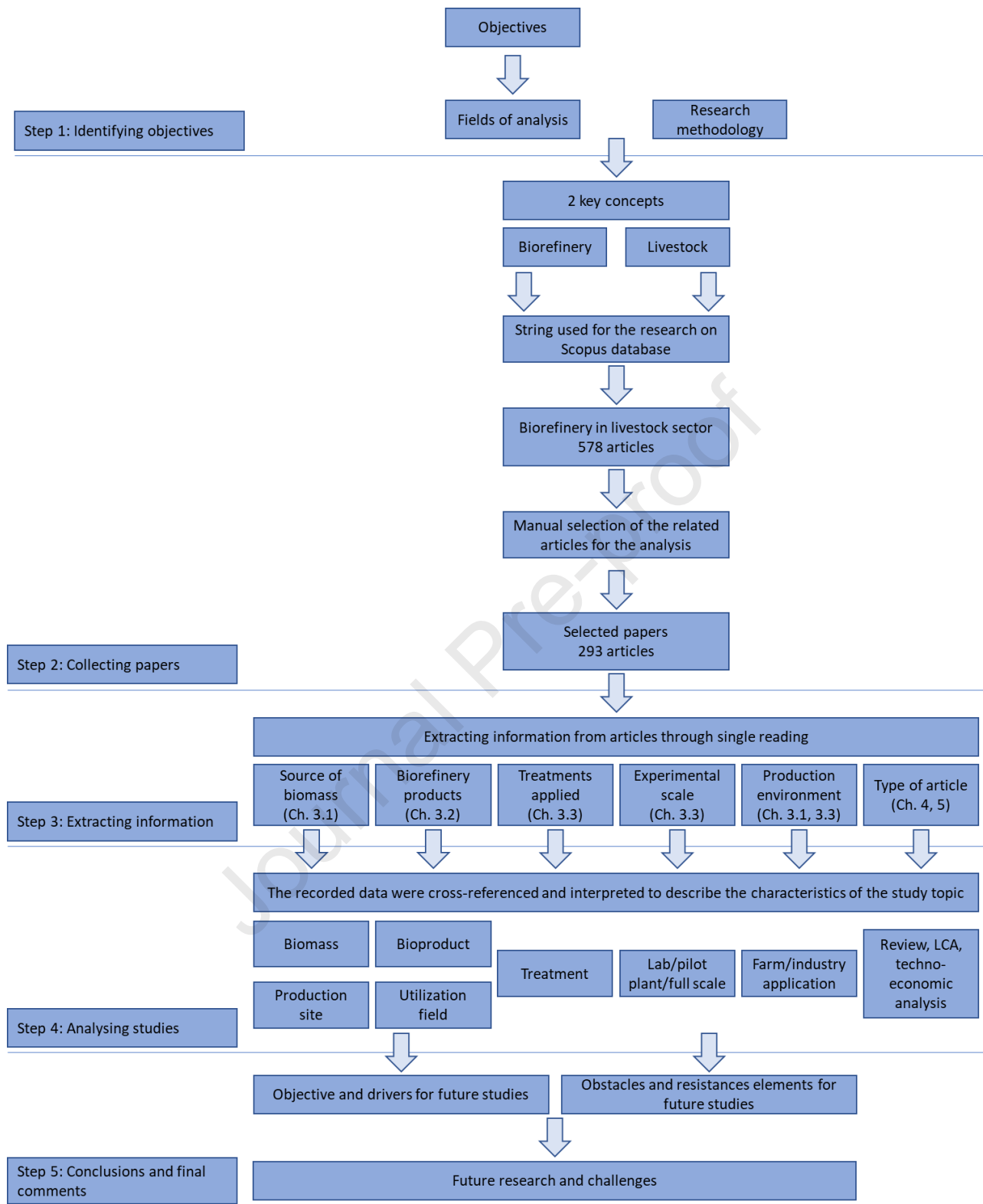
167 Articles that mentioned biorefinery only incidentally, without it being the subject of the article.

168 Articles that mentioned livestock breeding or certain animal species incidentally, without them being  
169 the subject of the study.

170 Conference articles with the same author and topic as a scientific article were included. In this case,  
171 the conference article was considered a duplicate.

172

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173

174 **Fig. 1.** Flowchart of the methodology used in this systematic review

175

176 The first categorisation of articles was based on the origin of the biomass (Section 3.1). Articles were  
 177 categorised according to the production site: agro-livestock farm, industry, or civil/urban area.

178 Although the main focus of this article was biomass from livestock farming, the study was completed

179 with the analysis of articles in which livestock farming was the destination of biorefinery activities.  
180 For biomass from livestock farming, the animals bred were also detailed to discuss the most common  
181 and important productions. Once the sectors of biomass production had been determined, the different  
182 types of biomass were described. Biomass was divided into manure, byproducts, main products,  
183 waste, and other specific types. These categorisations allowed for analysing the time course of  
184 scientific production by discriminating between the various sectors; it also made it possible to produce  
185 a series of considerations regarding the interactions between the biomass used and the processes  
186 implemented.

187 Once the origin and nature of the biomass used had been described, the analysis focused on the  
188 bioproducts obtained (Section 3.2). The first classification made it possible to describe the nature of  
189 the byproducts obtained; the main categories identified were biogas, biomethane, biofuels,  
190 bioethanol, bioplastics, microalgae, nutrients (fats, carbohydrates and proteins), fertilisers, and  
191 purified water. In addition to the total article count, the analysis made it possible to describe the  
192 temporal trends of the bioproducts obtained; this is useful information for hypothesising future  
193 biorefinery scenarios and trends. The categorisation of the nature of the bioproducts made it possible  
194 to define the production sectors for which the biorefinery processes are intended. A number of key  
195 destinations of use were also identified for this categorisation: animal feed, energy, fertilisers,  
196 pharmaceutical industry, chemical industry, manufacturing, and purified water. The description of  
197 the biomass of origin and the final bioproducts preceded the study of the processes used. The two  
198 pieces of information were then cross-referenced to determine which processes are most frequently  
199 associated with each type of biomass/bioproduct.

200 For research purposes, the analysed articles were classified according to the biorefinery process used  
201 and the production context in which the process occurred (Section 3.3). The biorefinery processes  
202 were grouped into the following categories: thermal, chemical, mechanical, biological, and anaerobic  
203 digestion. It was also recognised whether these processes took place in the laboratory, in pilot plants  
204 or on a full scale and whether the production context was agro-livestock farms or other industries.

### 205 3 Results

206 After filtering, the literature search identified 293 studies based on inclusion and exclusion criteria.

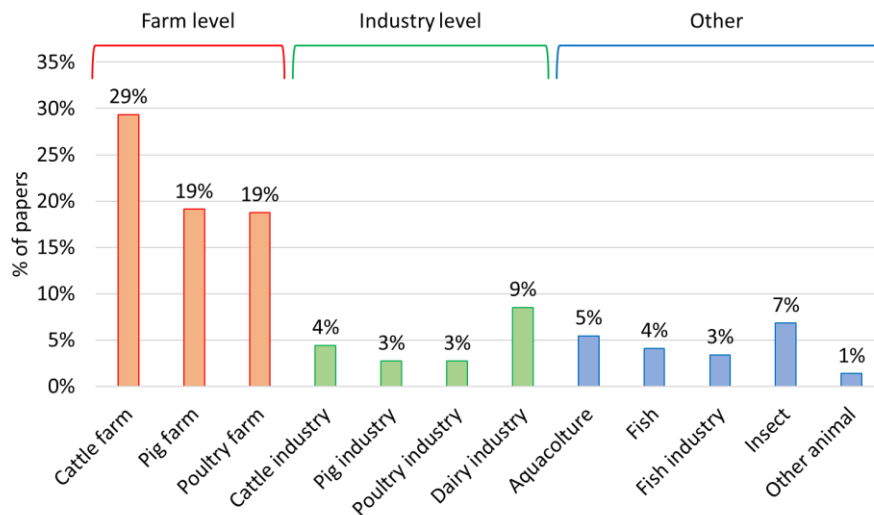
#### 207 3.1 Sources of biomass

208 To describe the state of the art of biorefineries, it is essential to carefully consider the biomass used.

209 In this review, 214 articles were analysed that utilise biomass from livestock (cattle, pigs, poultry,  
210 other animals), insects, aquaculture, and processing plants for products derived from these animals.

211 The remaining articles consider other biomasses, whether agricultural, civil, or industrial. For a better  
212 understanding of the information, in Fig. 2, the categories are grouped according to the area of origin  
213 of the biomass: “farm” for biomass produced directly in classic agro-livestock farms, “industry” if  
214 the biomass is produced in livestock production transformation processes, and “other” for particular  
215 livestock production activities. In scientific research, the most significant biomass contribution is  
216 cattle farming, with 86 articles, 29.4% of the total; pigs and poultry, both with 56 articles, 19.1%. The  
217 contribution of other animals, horses, sheep, etc., is much lower, 4 articles, or 1.4% of studies; this is  
218 due to the lower diffusion of these farms and to the smaller amount of biomass, mainly manure, that  
219 can be collected. The importance of cattle, pigs, and poultry is not limited to the livestock sector but  
220 also involves the processing industries. Of these, the most important is undoubtedly the dairy industry,  
221 which is mentioned as the source of biomass in 25 scientific contributions, corresponding to 8.5% of  
222 the total. The organic content of wastewater and waste from this industry makes these biomasses  
223 particularly suitable for biorefineries. A promising area in the next few years will be the breeding and  
224 utilisation of insects (Chapter 6). These can be used to process waste and other biomass and, above  
225 all, as a primary source of protein and other nutrients. These products are used to produce food for  
226 animals and, in the future, for humans. This analysis showed 20 articles, 6.8% of the total, in which  
227 insects were bred for biomass production.

228



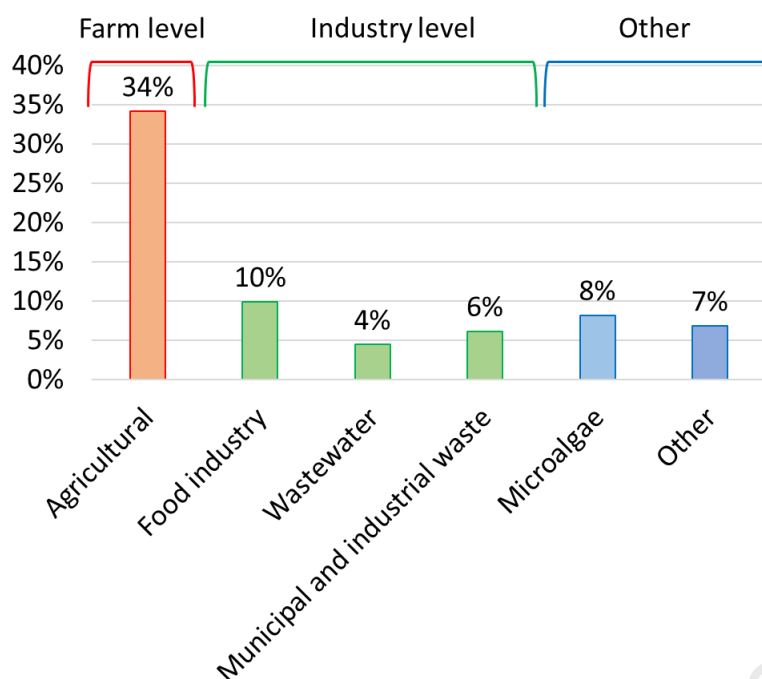
229

230 **Fig. 2.** Number of papers per origin of bioresources used (animal sector)

231

232 Among the non-livestock biomasses, the most common in this analysis were agricultural byproducts  
 233 (straw, cornstalk, pruning residues), with 100 papers, 34.1% of the total. This biomass is very often  
 234 used in combination with other biomasses, especially those from animal farming. It is mainly used  
 235 for energy purposes or the production of animal feed. Biomass from industry and settlements is less  
 236 used: 29 articles for food waste, 9.9%; 18 articles for civil and industrial waste, 6.1%; and 13 papers  
 237 for wastewater, 4.4%. Due to their characteristics, these biomasses often have to be treated differently  
 238 from byproducts and manure, so using them in combination is not always possible.

239 An interesting topic is microalgae; they are either used as biomass treatment, e.g., for removing  
 240 nutrients or harmful substances, or cultivated to produce biomass for protein, oils or carbohydrates.  
 241 This analysis found 24 articles dealing with this topic, 8.2% of the total number of articles. A more  
 242 specific search can verify the increase in research interest in this area; the number of articles published  
 243 on this topic in the biorefinery field rose from 23 papers in 2012 to 268 papers in 2022.



244

245 **Fig. 3.** Number of papers per origin of bioresources used (other sectors)

246

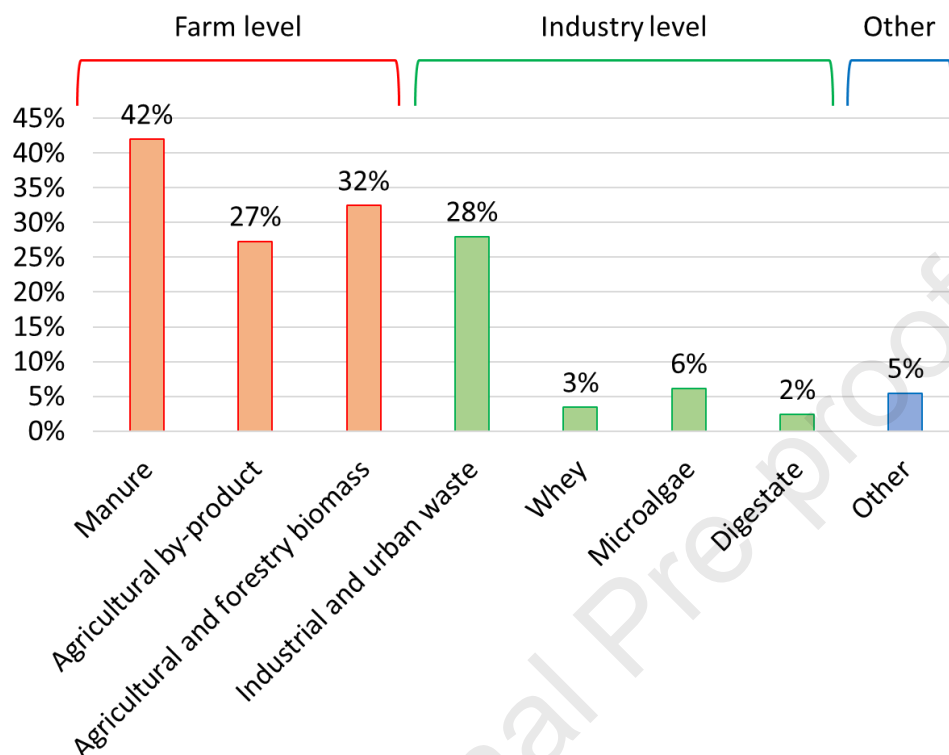
247 Once the areas of origin of biomass have been examined, the nature of the biomass itself can be  
 248 analysed (Table 1). In Table 1, in addition to the type of biomass, the environment of use, i.e., where  
 249 the biorefinery process takes place is also shown. The biorefinery can occur on the agro-livestock  
 250 farm, usually the same one where the biomass was produced, or in dedicated industries, where the  
 251 biomass is transported and processed.

252 In this analysis, most articles use animal manure, with 123 articles, 25.8%. However, manure is not  
 253 the only livestock biomass used: 10 articles used rumen, and 1 used urine. In addition, many articles  
 254 refer to poultry litter.

255 From the agro-forestry sector, 80 articles (16.8%) on agricultural byproducts and 95 articles (19.9%)  
 256 on agricultural and forestry biomass were identified in this analysis. In the first group, biomasses that  
 257 do not constitute the main product of cultivation were included, e.g., straw, clippings, and harvest  
 258 residues. The energy crops fall within the second group, namely, woody biomass harvested for the  
 259 biorefinery and hay and grass used as fodder.



260 Digestate was among the products used in 7 articles (1.5%). This result, although low compared to  
 261 the others, shows the importance of this product, not only as a natural byproduct of anaerobic  
 262 digestion but also as a primary product for other types of biorefineries.



263

264 **Fig. 4.** Number of articles per type of biomass used (see also Table 1)

265

266 Among the non-agricultural biomasses, the dairy industry's importance is demonstrated by the  
 267 explicit interest in whey as biomass for biorefinery applications, as demonstrated in 10 articles  
 268 (2.1%). This biomass is primarily used for energy production. However, there is no shortage of other  
 269 applications, such as the pharmaceutical, animal feed, and manufacturing industries.

270

271 **Table 1**

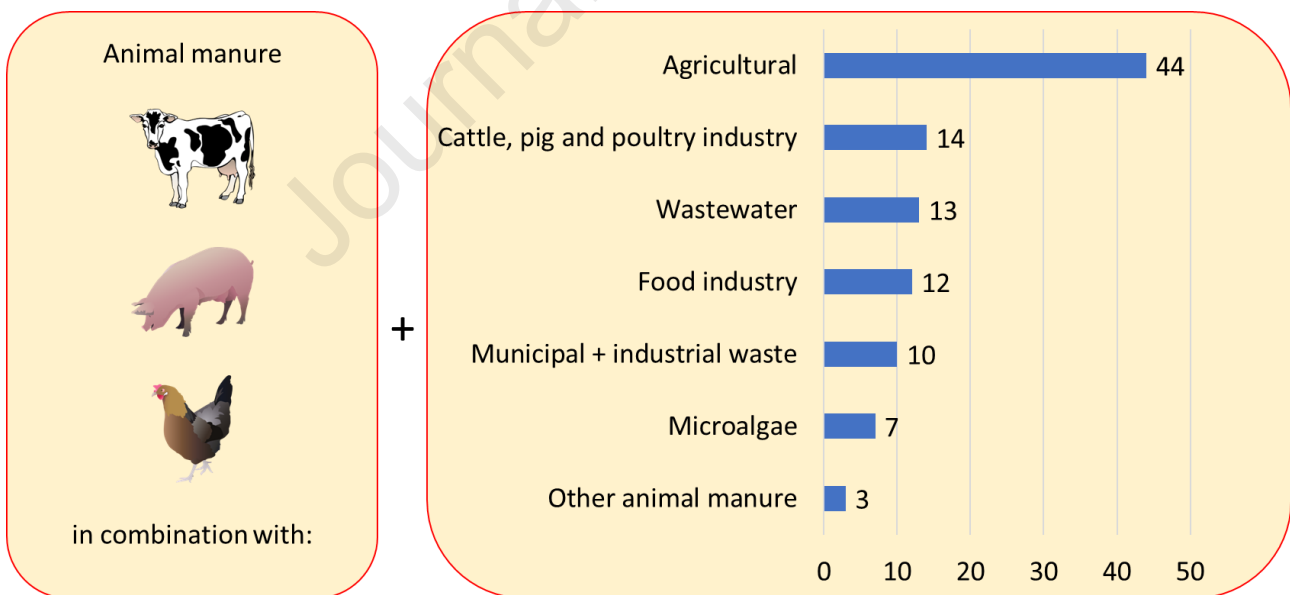
272 Number of articles per type of biomass used. The total is higher than the number of articles because more than  
 273 one biomass is used in many pieces of research.

Origin	Biomass	Number of articles and percentage (%)	Farm	Industry	Other
Agriculture	Manure	123 (42.0%)	57	62	17
	Agricultural byproduct	80 (27.3%)	29	48	15
	Agricultural and forestry biomass	95 (32.4%)	40	41	20
Industry	Whey	10 (3.4%)	0	10	0
	Industrial or urban waste	82 (28.0%)	13	69	6
	Microalgae	18 (6.1%)	2	12	3

Digestate	7 (2.4%)	3	6	0
Other	16 (5.5%)	5	11	1

274 The advantages of the combined use of biomass have been confirmed in numerous papers. This study  
 275 analysed biomass matrices that included animal manure to observe which biomasses were most often  
 276 combined with it (Fig. 3).

277 The biomass most frequently used combined with manure is agricultural waste and biomass, with 44  
 278 articles with both biomasses. This combination is also particularly frequent because it is the most  
 279 typical for anaerobic digestion in agriculture. In agro-livestock farms, it is common to use the two  
 280 matrices in combination to supply the digester. Another 15 articles combined biomass from livestock  
 281 farms with biomass from animal processing industries. In some cases, they are techno-economic or  
 282 LCA articles in which all biomass from a particular sector, farm animals in this case, is included. A  
 283 lesser weight in this analysis is found for wastewater (13 articles), biomass from the food industry  
 284 (12 articles) and civil and industrial waste (11 articles), probably due to their different origins than  
 285 manure.



286

287 **Fig. 5.** Biomasses in combination with cattle, pig, and poultry manure (n. of the articles)

288

## 289 3.2 Biorefinery products

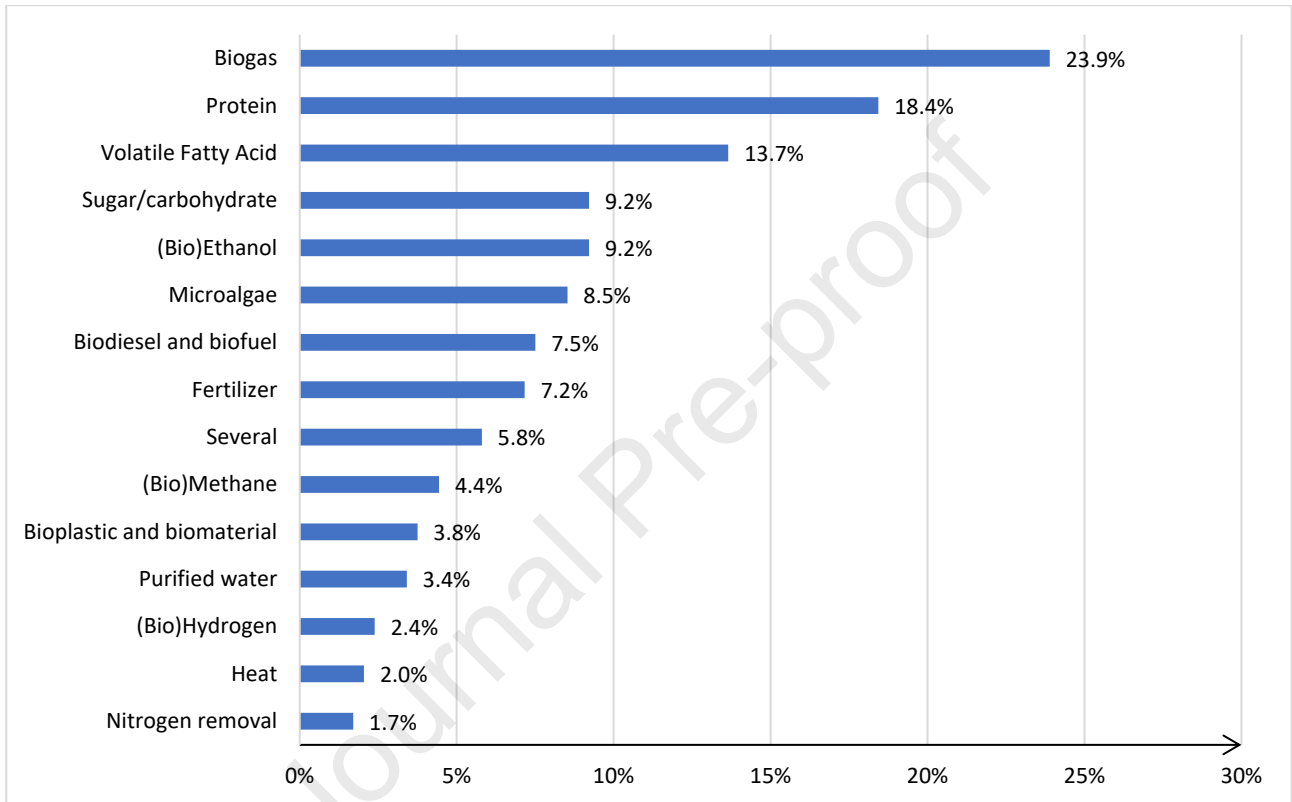
290 Once the biomasses were described, information about the bioproducts produced in the biorefinery  
291 process was extracted (Fig. 4). These confirm the analyses carried out earlier concerning the  
292 biomasses of origin and the types of treatments used.

293 Most of the processes are aimed at biogas production (70 articles, 23.9% of the total); in fact,  
294 anaerobic digestion is the most commonly used process. Closely related to biogas is the production  
295 of biomethane, which is examined in 13 articles, 4.4% of the total. In this analysis, the distinction  
296 between the two categories is based on what the author of the article identifies as the objective of  
297 their paper. Nevertheless, in bioenergy, two products have the same number of articles: ethanol and  
298 biodiesel (and biofuels); 22 articles. The first is used as an energy source and an animal feed additive.  
299 The topic of biofuels is currently crucial, and the increasing research trend confirms the interest of  
300 researchers in this topic (Fig. 5). The same applies to ethanol; this trend demonstrates the increased  
301 interest in this production. Lower values, but still worth considering, are reported for biohydrogen in  
302 7 articles and heat in 6 articles.

303 In addition to bioenergy, the other crucial area for byproducts is nutrients. Data on protein are notably  
304 interesting; this production is the topic in 54 articles. In many cases, it is the production of animal  
305 feed or supplements made from agricultural products or byproducts; in many other cases, the origin  
306 of the biomass from which the proteins are produced is insects, a sector that is overgrowing. In all  
307 cases, these articles focus their analysis on the sustainability of the livestock production chain. Indeed,  
308 reducing the energy, water and soil used for food production is a growing problem. Volatile fatty  
309 acids and carbohydrates were essential in 40 and 27 articles, respectively.

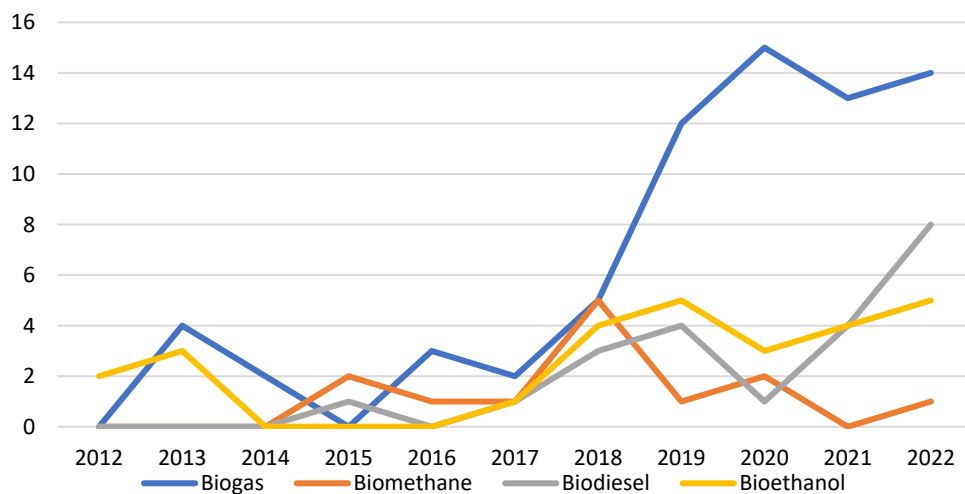
310 The production of fertiliser is significant, with 21 articles. This product is produced by anaerobic  
311 digestion in the form of digestate. However, in the articles cited in the count above, the reference to  
312 fertiliser by the research authors is explicit. This demonstrates the direct interest in this product and  
313 shows that it is not just a byproduct but constitutes the actual target of the study.

314 Some products are not considered in the analysis because they are irrelevant to the overall theme, not  
 315 sufficiently specified by the authors (e.g., in some papers, generic bioenergy production is  
 316 mentioned), or present with insufficient citations. Regarding the total of bioproducts obtained, the  
 317 same consideration applies to the biomass of origin: in many articles, several bioproducts obtained  
 318 are cited, so the total is higher than the number of articles considered.



319  
 320  
 321

**Fig. 6.** Number of citations by bioproducts produced in the biorefinery process in the articles considered.

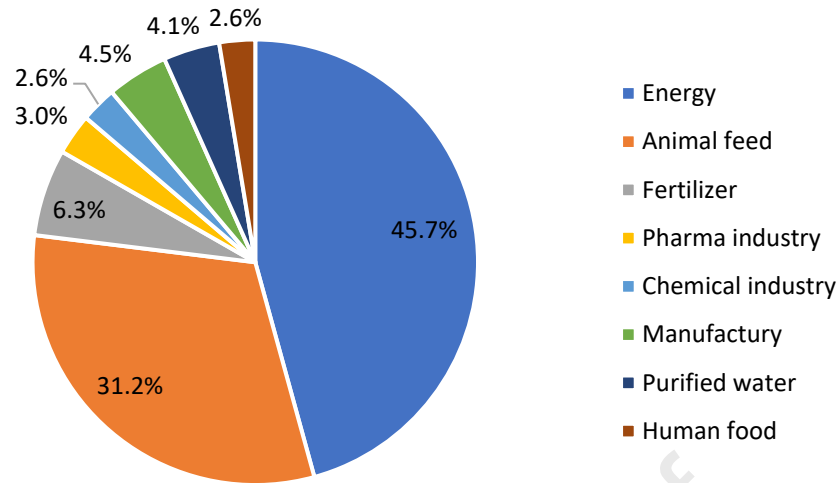


322

323 **Fig. 7.** Biogas, biomethane, biodiesel and biofuels and ethanol trends

324

325 The byproducts of the biorefinery were classified according to their intended use. The results confirm  
326 that biomass biorefineries are mainly directed towards energy production, with 123 articles (42.0%;  
327 Fig. 6). This condition has also increased in recent years (Fig. 5) and is likely to continue in the  
328 coming years, considering the emphasis on climate change and renewable energy. Another vital  
329 biomass utilisation sector is animal feed production, with 84 articles (28.7%). This sector is also  
330 growing, but with a slower trend; considering that much of the research in this area is techno-  
331 economic analysis and LCAs, this trend may be due to a relative maturity of the technology, which  
332 leads researchers and technicians to optimise existing solutions rather than to find new ones. In the  
333 agricultural and livestock sector, 17 papers, 5.8% of the total, concern fertiliser production. In most  
334 of these papers, fertiliser is only one of the bioproducts obtained; this proves the tendency of  
335 biorefinery research to work from a circular economy perspective, seeking to make the most of all  
336 available resources. Biomass produced in the livestock sector can also be used in various industrial  
337 sectors. In this analysis, the industrial sectors that used byproducts the most were the manufacturing  
338 sector (12 papers), the pharmaceutical industry (8 articles), and the chemical industry (7 papers). The  
339 production of food suitable for human consumption concerns a limited number of papers, 7 papers;  
340 these are review articles or processes that use agricultural or animal biomass to produce food suitable  
341 for both animals and humans.



342

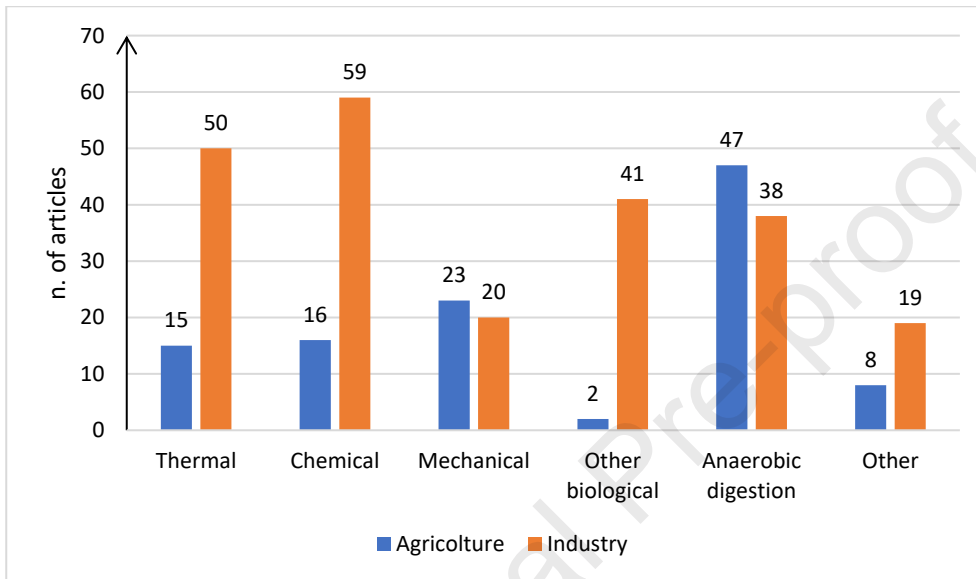
343 **Fig. 8.** Percentage of articles per economic/industrial sector of byproducts of the biorefinery

344

### 345 3.3 Treatments applied, experimental scale and production environment

346 The treatments used in the biorefinery processes of the investigated research papers were analysed  
 347 (Fig. 7). As expected, many techniques use several types of treatment, either sequentially or  
 348 simultaneously, or use treatments that can be included in more than one category.

349



350

351 **Fig. 9.** Type of treatment used in the biorefining process combined with the context of the process

352

353 As noted earlier in the analysis of the review articles, anaerobic digestion is the most widely used  
 354 treatment; 84 articles use it, almost a third of the total number of articles (28.7%). Anaerobic digestion  
 355 is particularly well suited to treating liquid or semiliquid biomasses such as manure and livestock  
 356 slurry. The widespread use of this treatment may be due to many factors: *i*) it can be applied to  
 357 different types of biomass, not only manure or agricultural byproducts but also urban and industrial  
 358 waste; *ii*) it allows biomass to be valorised from an energy point of view and as a byproduct produces  
 359 digestate, which is also a valuable product because it can be used as a fertiliser; *iii*) it can be installed  
 360 even in relatively small farms, agricultural or industrial, due to its relatively low costs and safe and  
 361 regular earnings (biomethane).

362 Chemical treatments were applied in 80 studies, 27.3% of the total. Many different treatments belong  
 363 to this category: alteration of pH, removal of metals, and composition or decomposition of organic



364 and nonorganic compounds. They are mainly used for civil and industrial waste, as they often contain  
365 substances incompatible with their valorisation and must therefore be pre-treated.

366 Thermal treatments are also widespread (70 articles, 23.9%). These treatments can enhance the  
367 biomass directly: combustion and gasification; or they can serve to prepare the biomass for other  
368 combined treatments, for example, they serve to heat it or keep it at a specific temperature. Thermal  
369 energy valorisation processes are well suited for biomass with low water content, such as agricultural  
370 residues or certain types of industrial waste. Applying these treatments to manure is associated with  
371 pretreatments such as drying or desiccation, or they are applied to composite matrices consisting of  
372 manure and other agricultural byproducts.

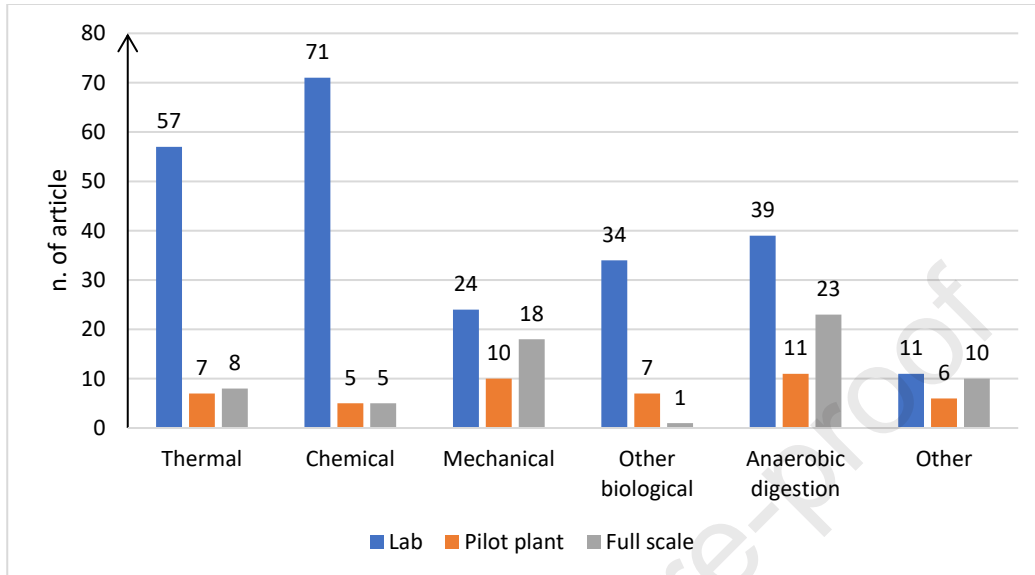
373 Mechanical treatments include all modifications to the size and constitution of the biomass. They  
374 include grinding, crushing and filtering. They are mainly applied to solid agricultural biomass  
375 intended to produce animal feed. Filtration is often used to pre-treat wastewater from livestock  
376 farming and civil and industrial wastewater. This set includes 47 articles or 16% of the total.

377 Biological treatments included 47 articles, 16% of the total. This broad category includes fungi,  
378 microalgae, and bacteria cultivation. These treatments are particularly suitable for treating liquid  
379 biomass, especially wastewater and runoff; they are used as pre-treatments for removing metals and  
380 other substances. Biological processes are a very heterogeneous category; even more varied is how  
381 they are used, as in most biorefinery processes in which they are present, they are used in combination  
382 with other treatments.

383 For a better description of the biorefinery processes, it is possible to cross-reference the data on the  
384 type of process used with the scale of application of the study. In Fig. 11, it is possible to observe  
385 how production processes are developed in the laboratory, in pilot plants and at full scale. In all cases,  
386 laboratory processes are the most common, but with significant differences. Thermal, chemical and  
387 biological processes are almost exclusively carried out in the laboratory; this suggests that these  
388 technologies and techniques are still in the experimental phase and will be the subject of future  
389 research and development. In contrast, mechanical processes and those using anaerobic digestion are

390 very often carried out in pilot or full-scale plants; these technologies are more mature and are being  
 391 tested to improve their performance, cost-effectiveness or reduce their environmental impact.

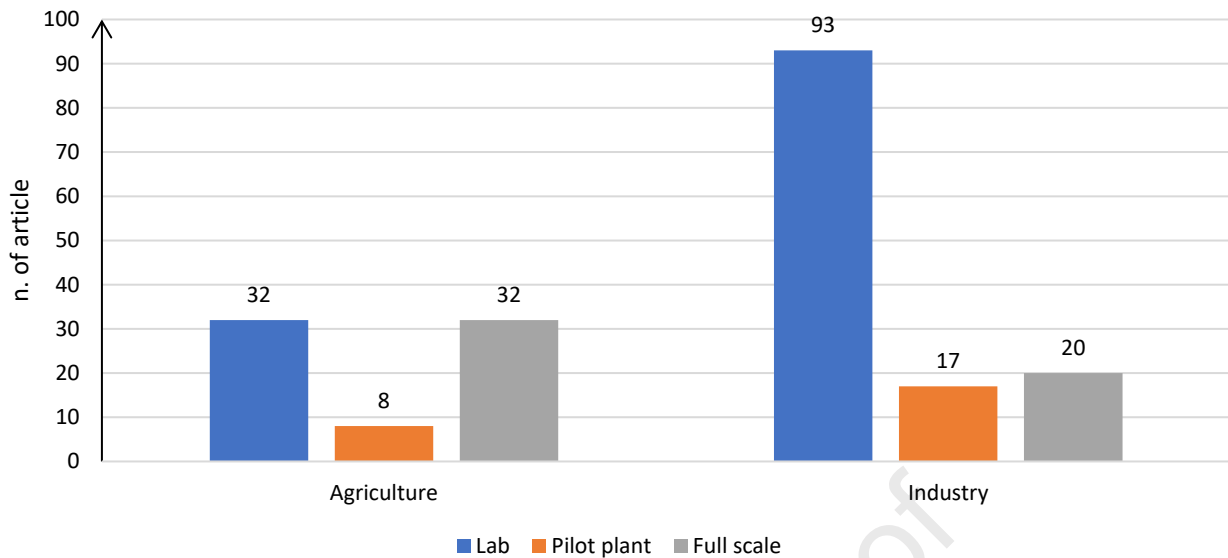
392



393

394 **Fig. 10.** Ways of setting up the research in the selected articles based on the biorefinery process type used  
 395

396 In general, biorefinery processes require the installation of major facilities with a relatively advanced  
 397 technological component. In the scientific literature, however, much research is carried out not in  
 398 full-scale facilities but in the laboratory. Information on the scale of application of the research was  
 399 collected (Fig. 8). Most of the articles, 61.9% of the total, i.e., 125 papers, are carried out in the  
 400 laboratory, i.e., in a very different environment from the real one, where the biorefinery will  
 401 eventually be applied once the technology is mature. The topics covered in these papers are generally  
 402 the most innovative, experimental ones.



403

404 **Fig. 11.** Ways of setting up the research in the selected articles

405

406 A much smaller proportion of paper, 25 papers, 12.4% of the total, is carried out in pilot plants; these  
 407 processes are generally situations with a more advanced degree of development. However, it is not  
 408 always easy to distinguish between pilot plants and the laboratory; the choice was made primarily  
 409 based on what the authors of the articles themselves stated in the methodology. Finally, 52 articles,  
 410 25.7% of the total, were carried out at full scale. Most of the technoeconomic studies and LCAs  
 411 belong to this group. Another group of papers that were carried out on a full-scale basis are those that  
 412 tested new diets for animals with food from the waste biomass biorefinery.

413 In Fig. 8, the methods used to conduct research in the published research are correlated with the  
 414 biomass processing environment (farming or industrial). The results demonstrate that processes  
 415 carried out in the agricultural context have good full-scale application, which are less applicable under  
 416 laboratory and pilot plant conditions; this proves that the biorefinery is in a more mature condition  
 417 and there is less innovation in this environment. The opposite is true for biorefinery processes  
 418 conducted in an industrial context; in this condition, there are fewer full-scale applications and more  
 419 at an experimental level, a sign that research is still at an experimental stage, with fewer real  
 420 applications. For some articles, it was impossible to determine whether they belonged to one of the

421 two categories because they were either review articles or LCAs, or the process was still in the  
422 experimental stage, and it was not possible to determine where it could be developed later.

423 These concepts will be addressed in Section 8, Fig. 9, where future research perspectives will be  
424 presented.

425

#### 426 3.4 Bioenergy production treatments

427 Based on the articles examined, a description of the treatments used for bioenergy production can be  
428 provided.

429 Bioenergy production using manure allows for the valorisation of waste products and avoids  
430 competition with food crops; the benefits of this practice have been documented in the scientific  
431 literature: mitigating pollution due to their management (Catenacci et al., 2022), decreasing costs  
432 related to the nitrogen disposal process (Femeena et al., 2022), and obtaining digestate valuable as  
433 fertiliser (Feiz et al., 2021). One of the most significant benefits of bioenergy is the possibility of  
434 providing different types of energy, depending on the biomass available and the needs of the energy  
435 system: electricity, heat, and fuel or biofuel, through the process of upgrading methane in liquid or  
436 gaseous form. However, it is also necessary to carefully identify the conditions that enhance the  
437 environmental sustainability of bioenergy production (Li et al., 2022) and to develop innovative  
438 technologies to improve anaerobic digestion.

439 Anaerobic digestion is the process of generating biogas through a series of biomass degradation  
440 processes (Holl et al., 2022). Biogas can be used to produce electricity, heat and biofuels (Ferrari et  
441 al., 2022). The most widespread technology in Europe allows combined heat and power production  
442 in the same plant (Rekleitis et al., 2020). Anaerobic digesters are connected to a gas engine to produce  
443 heat and electricity with an installed capacity typically ranging from a few tens of kWe to several  
444 MWe (Sganzerla et al., 2022). The heat generated can also be used for the needs of the farm facility,  
445 as well as, of course, being delivered to external users. Biogas can be upgraded to produce

446 biomethane, which can be injected into the natural gas transport grid or used as a vehicle fuel  
447 (Hamelin et al., 2021). Anaerobic digestion is an established technology and has been extensively  
448 studied (Rekleitis et al., 2020). Today, work on biogas is focusing on diversifying biomass,  
449 experimenting with new matrices and new combinations of feedstocks (Karki et al., 2021), increasing  
450 yields, improving process efficiency and refining resource management (Kassem et al., 2020).  
451 However, anaerobic digestion is not the only valid process for biomass valorisation; thermochemical  
452 valorisation processes cannot be overlooked among the most widespread and effective systems.  
453 Combustion is a thermochemical process for the utilisation of organic waste. This process is  
454 particularly suitable for biomass with a low moisture content (less than 20%) (Azwar et al., 2022).  
455 The hot gases obtained from the combustion process mainly comprise CO<sub>2</sub> and water vapour, and the  
456 steam generated can be efficiently used to power a steam turbine for energy generation (Bora et al.,  
457 2020). The end product of the combustion process is heat and other gases. This technology is  
458 particularly convenient in areas with a cold climate, where the high demand for heat makes the plants  
459 economically viable. Additionally, this technology is advantageous in developed countries with high  
460 population density, where the possibility of reducing the volumes and costs of managing the organic  
461 fraction of solid waste is significant (Odales-Bernal et al., 2021).  
462 Using combustion for livestock manure management is not a typical process, as this raw material has  
463 a high water content (Cavinato et al., 2017). However, drying, torrefaction and pelletisation processes  
464 can be adopted to utilise this matrix efficiently with this process (Khoshnevisan et al., 2021a), or  
465 manure can be used in combination with other biomasses (Karki et al., 2021). The combustion process  
466 produces many gases and ash: carbon monoxide, nitrogen oxides and acid gases such as sulfur  
467 dioxide. Because of these emissions, phosphorus and potassium recovery technologies from livestock  
468 manure intended for combustion have recently become widespread (Awasthi et al., 2019).  
469 Technologies for recovering nutrients from waste and byproducts, such as livestock manure, are  
470 becoming increasingly common. Manure, especially the liquid fraction, contains significant amounts  
471 of nitrogen and phosphorous (Cavinato et al., 2017). In areas with intensive livestock activity, this

472 can cause severe problems of oversupply of these nutrients as fertilisers and lead to soil acidification  
473 and eutrophication (Møller et al., 2022). The recovered nutrients can be further exploited by  
474 producing biomaterials and bioproducts. Among the most popular recovery processes are ammonia  
475 stripping, chemical precipitation, ion exchange, membrane separation, and thermal treatments.

476 Ammonia stripping takes place in stripping towers; in these facilities, the nitrogen available in the  
477 liquid substrate passes into the gas phase in the form of  $\text{NH}_3$ . Ammonia stripping is a relatively simple  
478 process, but attention must be paid to pH control and aeration. Another method of nutrient recovery  
479 is the precipitation of struvite, which allows the recovery of nitrogen and phosphorous. The most  
480 significant advantage of struvite formation is the low energy demand, while the low percentage of  
481 recovered nitrogen is the main drawback (Vaneckhaute et al., 2019).

482 Membrane technologies, such as reverse osmosis, nanofiltration, membrane distillation, and  
483 electrodialysis, have excellent performance in recovering resources from liquid biomass. These  
484 technologies can be divided into pressure and non-pressure technologies. Pressure-based membrane  
485 filtration requires an energy of 4-6 kWh/m<sup>3</sup> and an operating cost of 4-13 €/m<sup>3</sup> in operational plants  
486 (according to a study conducted on several situations in different countries of the world)  
487 (Khoshnevisan et al., 2021b). Filtration and reverse osmosis are classified among the pressure  
488 membrane technologies. Generally, these technologies are unsuitable for manure treatment, as  
489 manure contains a high value of organic matter and total solids (TS). However, they are well suited  
490 to treating digestate or the liquid fraction of animal slurry (Khoshnevisan et al., 2021b). The choice  
491 between different pretreatments for nutrient removal and valorisation systems (bioenergy,  
492 bioproducts) depends on the biomass characteristics and environmental requirements.

493

494

#### 495 **4 Review article analysis**

496 The first analysis focused on review articles. Biorefineries involve numerous topics; for this reason,  
497 research has followed various directions that are also very different. Consequently, many authors  
498 have periodically reviewed scientific advances in this multidisciplinary field with numerous review  
499 articles. These articles were analysed, and key themes and features were derived.

500 To include this work within the framework of previous reviews, the most significant review articles  
501 in animal livestock were summarised first (Table 2). The application of biorefinery processes to  
502 livestock manure has mainly concerned energy production. Among the first authors to summarise the  
503 scientific conclusions, Awasthi et al. (2019) and Khoshnevisan et al. (2021b) analysed both biogas  
504 and digestate production for agronomic purposes. They considered manure produced by different  
505 types of animals, cattle, pigs, and poultry, and concluded that livestock manure management could  
506 replace 60–75% synthetic fertiliser with some extra gain in bioenergy and nutrients. Other authors  
507 have directed the review towards a particular species of animal, e.g., cattle (Mandavgane and  
508 Kulkarni, 2020), pigs (Walowsky, 2021), or poultry (Alba Reyes et al., 2021). However, the use of  
509 manure is not limited to energy production: Zhu and Hiltunen, 2016 and Zhu et al., 2021, summarised  
510 the state of the art regarding the cultivation of microalgae with farm manure. The results demonstrated  
511 that pretreatment of dry matter before conversion is required to obtain a high sugar yield for microbial  
512 fermentation because, in general, dry matter substrates have lower carbohydrate content relative to  
513 other substrates. Different pre-treatments showed their advantages and disadvantages regarding the  
514 efficiency, formation of inhibitors, energy consumption, and process costs.

515 One of the essential aspects of the research was the integration of livestock manure with other  
516 byproducts of agricultural origin. This combination fully meets the need to develop a circular  
517 economy: within the same production centre, the agro-livestock farm, various productions can be  
518 combined to exploit the characteristics of the respective biomasses produced. In Li et al., 2012,  
519 Rekleitis et al., 2020, and Mendes et al., 2022, the results of integrating farm waste with agricultural  
520 byproducts are analysed. In Catenacci et al., 2022 and Nzeteu et al., 2022, the analysis is directed at



521 the results of integrating manure with food waste. With a combination of these biomasses, the  
522 integration of waste management in agricultural and civil/urban areas is realised. Moreover,  
523 Catenacci et al., 2022 demonstrated the advantages of combining the digestate as a fertiliser and its  
524 energetic valorisation to produce char.

525 An interesting aspect is the management of animal manure in combination with insects. A fascinating  
526 examination of this area is provided by Rajeswari et al., 2021, who analysed gut microbial community  
527 enrichment strategies and molecular characterisation techniques to understand microbial community  
528 dynamics of several insects and ruminants for second generation production of biofuels and  
529 chemicals. According to the authors, to strengthen the perspective of the second-generation biofuels  
530 industry, implementing a centralised market is required to provide homogenous supply routes and an  
531 integrated bioprocess strategy for the cost competitiveness of these biofuels.

532 Anaerobic digestion is the most widely used process for the treatment of biomass. Numerous authors  
533 have conducted studies applying this technique, and multiple review articles have summarised them;  
534 interest in this area is still high. For example, Pelaez-Samaniego et al. (2017) and Sevillano et al.  
535 (2021) summarised the results of anaerobic digestion of manure, particularly cattle manure, in  
536 combination with agricultural products, showing the advantages of using these biomasses in  
537 combination on heavy metal accumulation, increased soil salinity, phytotoxicity, and ecotoxicity. The  
538 study by Karki et al. (2021) is on this topic; they examined the state of the art of anaerobic digestion,  
539 in particular showing the limitations of mono-digestion, compared with the advantages of systems  
540 that use multiple substrates: synergistic interactions via balance of nutrients, supplementation of trace  
541 elements, dilution of toxic and inhibitory compounds, and promotion of microbial diversity to  
542 maintain diverse microbial communities during long-term codigestion.

543 Over the years, research interest has grown in one particular sector, the dairy industry. The increase  
544 in research in this area has led to a rise in the frequency of publication of review articles: one article  
545 in 2018 (Chandra et al., 2018), two articles in 2020 (Asunis et al., 2020; Sebastián-Nicolás et al.,  
546 2020) and 2021 (Carvalho et al., 2021; Zandona et al., 2021) and three articles in 2022 (Gottardo et

547 al., 2022; Kumar Awasthi et al., 2022; Sar et al., 2022). This type of industry produces a significant  
548 amount of biomass in liquid form, with enough organic matter to generate considerable energy.  
549 The growing interest in the circular economy has increased the focus on the economic and  
550 environmental consequences and costs of products and processes. In response to the need to optimise  
551 investments and reduce the consumption of resources and the production of pollutants, many authors  
552 have carried out techno-economic analyses of processes and LCAs of products. This scientific  
553 production has also covered less available but essential products. For example, Odales-Bernal et al.,  
554 2021 summarised research on poultry litter exploitation to propose optimised systems for exploiting  
555 this biomass and promoting its use; they concluded that the treatment of poultry litter in biorefineries  
556 in Cuba would have a positive impact on the economy through income generation and savings  
557 resulting from reductions in imports (i.e., fossil fuels and agrochemicals), employment creation,  
558 improved living conditions and development in rural communities. Awasthi et al., 2022 summarised  
559 the scientific findings regarding the environmental impacts of livestock manure management; through  
560 the analysis of various life cycle assessments and technoeconomic assessments, they composed a  
561 state-of-the-art picture and indicated exciting perspectives for research and regulations and policies  
562 in the field.  
563

564 **Table 2**  
565 Analysis of previous review articles

Topic	Year	Biomass used	Bioproduct(s)	Treatment(s)	Reference
Valorisation potential of various sustainably sourced feedstocks, particularly food wastes and agricultural and animal residues	2022	Food waste, grass and manure	Biogas, bioproducts, VFA	n.s.	(Nzeteu et al., 2022)
Anaerobic digestion integration with pyrolysis/HTC, digestate as feedstocks for char production	2022	Food waste, agricultural byproducts and manure	Biogas	n.s.	(Catenacci et al., 2022)
Techno-economic assessment and life cycle assessment of livestock manure management operation in the context of their economic and environmental sustainability	2022	Cattle, pig, poultry farm manure	Biogas, nutrient recover	AD	(Awasthi et al., 2022)
Bibliographical survey of biomass generated in Brazilian agroindustry as a cosubstrate for energy production	2022	Agricultural byproducts, cattle, pig and poultry manure	Biogas	AD	(Mendes et al., 2022)
Enrichment strategy of gut microbial community and its molecular characterisation techniques to understand the holistic microbial community dynamics.	2021	Insects and ruminant manure and waste	Biofuel	n.s.	(Rajeswari et al., 2021)
Review of different types of bioenergy production from dairy manure and provided a general overview for bioenergy production	2021	Cattle manure	Biogas, bioethanol, biohydrogen, microbial fuel cell, lactic acid	AD	(Zhu et al., 2021)
Sustainable pathways to maximise the PL valorisation process, and showing the advantages of reforming poultry farms into biorefineries in Cuba	2021	Poultry manure	Several energy products	Thermochemical processes and AD	(Odales-Bernal et al., 2021)
Current leachate processes that could be applied as a previous step during the AD of CM, in addition to deep on the state of the art of HRAR using CM leachate as a liquid substrate for AD	2021	Poultry manure	Biogas	AD	(Alba Reyes et al., 2021)
Systems and technological variants of biogas production	2021	Pig manure	Biogas	AD	(Walowsky, 2021)
Most employed manure management technologies, challenges, sustainability, environmental regulations and incentives, improvement strategies perspectives	2021	Livestock manure	Several products: energy, fertiliser	n.s.	(Khoshnevisan et al., 2021b)
Biorefinery biomass technology, energy production technology, production of biofuels, and new materials from waste biomass at the behest of the circular economy and bioeconomy	2020	Agricultural and livestock waste	Bioenergy	n.s.	(Rekleitis et al., 2020)

Physicochemical composition and valorisation of cow urine and dung.	2020	Cattle manure	Biogas, digestate	AD	(Mandavgane and Kulkarni, 2020)
Review of organic manure biorefinery models towards sustainable circular bioeconomy	2019	Livestock manure	Biogas, digestate	AD	(Awasthi et al., 2019)
Microalgal cultivation with livestock waste compost for continuous production of multiple bioproducts	2016	Livestock manure	n.s.	Microalgae cultivation	(Zhu and Hiltunen, 2016)

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## 567 **5 LCA papers and techno-economic analysis**

568 Research in biorefineries has not only focused on chemistry or the physics of processes (Li et al.,  
569 2022). Often, industries intend to use established technologies but must verify the technical feasibility  
570 and economic viability of applying specific methods (Rhee et al., 2021). For this reason, research has  
571 focused on the technical-economic feasibility instead of the experimental-scientific feasibility.

572 Most of the technical analyses relate to processes involving cattle breeding. However, in contrast to  
573 other studies, in addition to the interest in products and processes that utilise manure, a considerable  
574 interest of researchers can be observed in the production of biomass destined for cattle farming,  
575 particularly for food production. The research of Demichelis et al., 2019 and Kassem et al., 2020  
576 belongs to the first group of studies. These authors set the analysis on a large scale, calculating the  
577 environmental impact of cattle manure uses for large study areas. Demichelis et al., 2019 developed  
578 a method for the environmental and technical quantification of biowaste management in Italy.  
579 Through a geolocation system of waste and knowing its characteristics, it is possible to determine the  
580 best process for its valorisation.

581 Interestingly, the same authors (Demichelis et al., 2019) later extended the analysis to a European  
582 level, testing it on a larger scale. Kassem et al., 2020 implemented a system combining various  
583 valorisation processes to quantify the expense and economic return of utilising the manure produced  
584 by 397,000 cattle in New York State. On the other hand, Joglekar et al., 2020 focused their studies  
585 on one particular process and quantified the sustainability of a biorefinery using cattle manure,  
586 applying a sustainability index based on a multicriteria analysis. Another innovative approach was  
587 studied by Rhee et al., 2021, who combined manure with microalgae for energy production. The  
588 utilisation of microalgae is also confirmed as a promising area from a technoeconomic point of view;  
589 this supports the idea that extensive applied research will have to be devoted to this area in the future.  
590 Finally, the use of agricultural biomass for cattle feed production was discussed in two papers, both  
591 from Brazil and both using sugar cane as a crop for nutrient production. Junqueira et al., 2018 used a

592 digital architecture to simulate an ethanol production process for cattle feed. Additionally, de Souza  
593 et al., 2019 used digital simulation models; in this case, cattle pasture was integrated with sugarcane  
594 cultivation, and the possible savings in CO<sub>2</sub> emissions using this system were simulated.

595 Of course, cattle are not the only source of manure investigated by researchers. Pig manure  
596 management can become a significant issue, especially since this type of livestock farming tends to  
597 be concentrated in specific geographical areas. These analyses were carried out by Vaneekhaute et  
598 al., 2019 in Canada and Lee and Tsai, 2020 in Taiwan; these authors used data libraries to quantify  
599 the volumes of biomass generated by pig farming and the environmental benefits of proper  
600 management. The agronomic aspect is addressed in the work of Tampio et al., 2019, who studied the  
601 effects on the phosphorous and nitrogen cycle of fodder cultivation in combination with pig farming:  
602 animal feeding, soil fertilisation and anaerobic digestion are the steps/processes in which the two  
603 biomasses are integrated. The high energy value of poultry manure makes this biomass particularly  
604 suitable for thermal processes. Tao and You, 2020, and Bora et al., 2020, studied this topic; the first  
605 one from a geographical point of view, identifying the most advantageous supply chains in New York  
606 State; the second one by comparing alternative processes for energy valorisation in nine plants and  
607 calculating the respective costs and gains.

608 As previously described, anaerobic digestion is the most widely used process for biomass  
609 valorisation. In this area, technoeconomic analyses follow three approaches: *i*) the planning of  
610 interventions, with the forecasting of costs and economic and environmental gains from the  
611 construction and use of the plants (Bramstoft et al., 2020); *ii*) the verification of actions taken,  
612 especially of legislative and regulatory initiatives in particular geographical/administrative areas  
613 (Curry et al., 2018); and *iii*) the review of the literature, with a periodic update of the state of the art  
614 of the technology (Sevillano et al., 2021).

615 Studies that did not use biomass from livestock farming but used various biomasses to produce  
616 bioproducts for animals, mainly feed, were analysed. It was illustrated that the use of agricultural  
617 byproducts for the production of animal feed is widespread; the analysis showed that in this area,

618 many technoeconomic studies were directed towards the evaluation of processes for ethanol  
619 production (Turner and Saville, 2022; Vaskan et al., 2018; Weinwurm et al., 2013). In other research,  
620 ethanol production combines agricultural byproducts with livestock manure. Li et al., 2022 and Capaz  
621 et al., 2021 evaluated the viability of processes that use a mix of animal and plant biomasses to  
622 produce ethanol for use as biofuel, the first for maritime transport and the second for air transport.  
623 It is interesting to note the work of Guilayn et al., 2020, who studied the benefits of using digestate  
624 as a fertiliser and a thermal energy source. The study demonstrated the need to analyse the costs and  
625 gains of each step of the biorefinery to set up an efficient circular economy of biomass.

626 The large number of processes and technological solutions that research and technology have made  
627 available allows a certain freedom of choice in defining the tools available to achieve production  
628 goals. For this reason, in addition to scientific experimentation and technical-economic  
629 characterisation, to choose one process or product over another, it is necessary to compare alternatives  
630 based on their overall emissions over their entire life cycle (Table 3). Life cycle assessment has  
631 precisely this objective, and numerous authors have applied this concept in the biorefinery of products  
632 from and for livestock farming.

633 The importance and spread of the dairy industry and the high volumes of wastewater produced, with  
634 the associated costs, have led many authors to evaluate, from an environmental point of view, several  
635 alternatives for their treatment. Kopperi and Mohan, 2022 assessed the feasibility of a biorefinery  
636 process that uses wastewater from the dairy industry to produce microalgae; the microalgae are then  
637 used to produce energy. In this way, wastewater, a waste product, is valorised and undergoes an initial  
638 purification treatment. An interesting example of a complete life cycle is that offered by Ivanov et  
639 al., 2022, who analysed the combined life cycle of the dairy industry, the wastewater supply chain  
640 from production industries to treatment sites, and biodiesel production from the same.

641 Ethanol production remains of interest and topicality. Indeed, numerous researchers have analysed  
642 and compared alternative processes to determine the best conditions for production. In Brazil, land  
643 consumption for sugarcane ethanol production, cattle breeding and forest conservation is particularly

644 important given the scale of the uses mentioned above; the topic was investigated by de Souza et al.,  
645 2019, who studied sugarcane ethanol production in combination with cattle breeding to avoid the  
646 consumption of forest area. In Europe ethanol production is linked to sugar beet; in Demichelis et al.,  
647 2020, this possibility was compared with the use of cattle manure, agricultural byproducts and  
648 municipal solid waste. Interestingly, while sugar beet is the most economically viable biomass,  
649 animal manure is environmentally preferable. The results showed how important it is to define the  
650 objectives of the processes, as calculations alone are insufficient to determine the absolute best  
651 alternative.

652 Cow manure is the most widely used biomass in animal husbandry, which is also demonstrated in  
653 LCA analyses. Usually, studies consider this biomass in combination with others to improve its  
654 performance. Among the others, the use of algae is one of the most promising choices: the production  
655 of biodiesel with different mixes of microalgae and cattle manure was studied by Maranduba et al.,  
656 2015; the results showed the advantages of this choice, as in scenarios where the two biomasses are  
657 used in combination, a reduction in GHG emissions of 53.6% and 63.8% is achieved, depending on  
658 the process used. Manure can also be used in combination with agricultural biomass. For example, in  
659 Vega et al., 2019, manure is used together with grape pomace to produce biogas and biomaterials;  
660 the comparison showed that combined bioenergy-biomaterial production is the most cost-effective  
661 because it makes full use of the available resources.

662 Remaining in cattle livestock, several authors have tested the impact of different animal diets,  
663 combining various types of biomasses from agricultural and other activities. Patterson et al., 2021  
664 compared the use of hay for cattle feeding with its use to produce certain types of materials; in this  
665 way, they could estimate the environmental benefit of reducing meat consumption and the consequent  
666 use of hay for other processes. Even more specific is the topic addressed by Taelman et al., 2015,  
667 who compared the emissions of soya-based animal feed production and the same production based  
668 on algae. The results indicated that seaweed has a significantly higher carbon footprint; however, in



669 their discussions, the authors attributed this result to the economies of scale present for soya but not  
670 for seaweed, the cultivation of which is still not widespread on an industrial scale.

671 In addition to cattle manure, other livestock biomasses are used. In Parajuli et al., 2018, cattle manure  
672 and pig manure were combined in different mixes for bioenergy production; the authors found that  
673 codigestion is the solution with the lowest emissions. The research of Moretti et al. (2018), who  
674 combined organic solid waste with cattle manure, should also be mentioned regarding this topic; the  
675 results again confirmed that codigestion is the best solution to reduce GHG emissions. In recent years,  
676 the exploitation of insects for energy and biomaterials has been gaining ground. Rosa et al., 2020  
677 quantified the emissions from producing biomaterials derived from proteins extracted from black  
678 soldier fly larvae; the larvae grew on poultry manure. As in the case of cattle, the authors were  
679 interested in assessing the environmental impact of alternative diets, which allow animals to be fed  
680 using waste biomass while limiting land use for dedicated crops for other livestock farms, particularly  
681 pigs. LCAs of two grass- and grain-based diets were proposed by Cong and Termansen, 2016 to  
682 reduce the environmental impact of pig farming, which is a significant problem in Denmark. Their  
683 results showed that the protein-based diet from the grass biorefinery reduces the feed cost, produces  
684 additional gains for the biorefinery and reduces nitrogen leaching. More recently, Møller et al., 2022  
685 proposed a similar study on the sustainability of pig production based on a diet containing yeast as a  
686 protein source. This yeast-based diet is compared to a classic soy-based diet. The environmental  
687 impacts of the two systems were compared using LCA; the results proved that replacing soya with a  
688 yeast-based diet reduces environmental impacts in terms of biodiversity loss and climate change. This  
689 research allowed a comparison of the different systems also considering land consumption and  
690 showed that the biorefinery provides significant resource savings, reducing the impact on natural and  
691 forest areas.

692

693 **Table 3**  
694 Life cycle assessment process for manure management sustainability

Topic	Biomass used	Scale	Sector	Bioproduct(s)	Object of LCA	Main results	Reference
Optimal design of a sustainable combined supply chain to produce biodiesel	Dairy manure	Full scale	Agricultural	Biodiesel	Biodiesel from dairy waste	Total cost of the optimal supply chain: 10,593,364 \$	(Ivanov et al., 2022)
Integration of dairy wastewater treatment, hydrothermal liquefaction of defatted algal biomass, and acidogenic process in a semisynthetic framework	Dairy wastewater	Pilot plant and full scale	Industry	Microalgae for pharma industry	Microalgae from dairy waste	Total bio-H <sub>2</sub> production of 231 ml/g of TOC with a 63% treatment efficiency.	(Kopperi and Mohan, 2022)
Analysis of the sustainability of pig production based on a diet containing yeast as a protein source	Wood	Lab and pilot plant	n.s.	Yeast to produce sugar	Yeast to produce sugar	Feed production causes: 64% of climate change, 70% of climate change and 100% of the land occupation	(Møller et al., 2022)
Evaluation the utilising grass to produce high value products, specifically PHA biopolymers, in a biorefinery approach	Grass	Full scale	n.s.	Protein	Feed for cattle from grass	A total of 30,000 t of fresh grass would yield approximately 403.65 t of dried biopolymer granules	(Patterson et al., 2021)
Technical, economic and environmental assessment of bioethanol production from waste biomass	Sugarcane, potatoes, rice straw, cattle manure and OFMSW	n.s.	Agricultural and industry	Bioethanol	Ethanol from different agricultural and livestock manure	0.19 kg of bioethanol per kg of cattle manure	(Demichelis et al., 2020)
Comparison of the environmental sustainability assessments of different extraction/fractionation procedures	Poultry farm	n.s.	Industrial	Protein	Bioproducts from larvae of BSF from poultry manure	The enzymatic approach resulted for the 31.87% more environmentally impacting with respect to the chemical method.	(Rosa et al., 2020)
Examining environmental impacts arising from technology-to-region compatibility, the framework is applied to two biorefinery alternatives, treating a mixture of cow manure and grape marc.	Cow manure and grape marc	Full scale	n.s.	Biogas and PHA	Biogas or biomaterials from cattle manure	1.59 and 1.40 person-equivalent of avoided GWP per ton of treated feedstock per day in France and Oregon, respectively	(Vega et al., 2019)

Techno-economic and environmental feasibility of sugarcane ethanol and cattle integration	Sugarcane	n.s.	n.s.	Bioethanol	Ethanol from sugarcane for cattle feeding	0.9 kg CO <sub>2</sub> eq per kg of ethanol; 0.5 kg CO <sub>2</sub> eq per kg of sugar and 0.08 kg CO <sub>2</sub> eq per kWh of electricity produced	(de Souza et al., 2019)
Effect of time on bioenergy production from dairy manure and associated variation in energy demand and GHG emission	Cattle manure	Full scale	Agricultural	Biogas	Bioenergy from cattle manure from different resident time	28–35 kg CO <sub>2</sub> /GJ of bioenergy produced	(Chowdhury et al., 2018)
Evaluate the environmental impacts of a combined production of suckler cow calves and Pigs, calculated in terms of their live weight	Cattle and pig manure	Full scale	Agricultural	Biomethane	Three bioenergy production systems from cattle and pig manure	1 kg of cattle manure and 1 kg of pig manure produce 19.6 kg CO <sub>2</sub> eq for carbon footprint	(Parajuli et al., 2018)
LCA of two scenarios for the biological treatment of local organic municipal solid waste and pig manure in the Netherlands	Organic municipal solid waste and pig manure	Full scale	Industrial	Biogas	AD of two diets with OMSW and pig manure	0.17 Mt CO <sub>2</sub> eq./yr for Scenario 1 and 0.16 Mt CO <sub>2</sub> eq./yr for Scenario 2	(Moretti et al., 2018)
LCA of three cattle manure biorefineries: first and second scenarios, the biogas is used for electricity and transportation; third scenario, the biogas is recycled back to the systems	Cattle manure	Full scale	n.s.	Biogas	AD of two diets with macroalgae and cattle manure	The life cycle of biogas production from cattle manure is 2017 mPt	(Giwa, 2017)
Comparison of the economic and environmental effects of producing the pig feed using two feeding systems	Grass	Full scale	Agricultural	Protein	Two pig feed with grass and cereals	To produce 1 ton of pork, with the cereal-based feeding system roughly 0.61 ton barley and 0.2 ton soya are needed	(Cong and Termansen, 2016)
Analysis of the biodiesel production system via dry-route, based on <i>Chlorella vulgaris</i> cultivated in raceways, by comparing the GHG-footprints of diverse microalgae-biodiesel scenarios	Cattle manure	n.s.	n.s.	Biodiesel	Five mix of microalgae and cattle manure for biodiesel production	The C1 and C2 scenarios presented GHG emissions of 5.10 and 4.88 t CO <sub>2</sub> -eq/t biodiesel, respectively	(Maranduba et al., 2015)

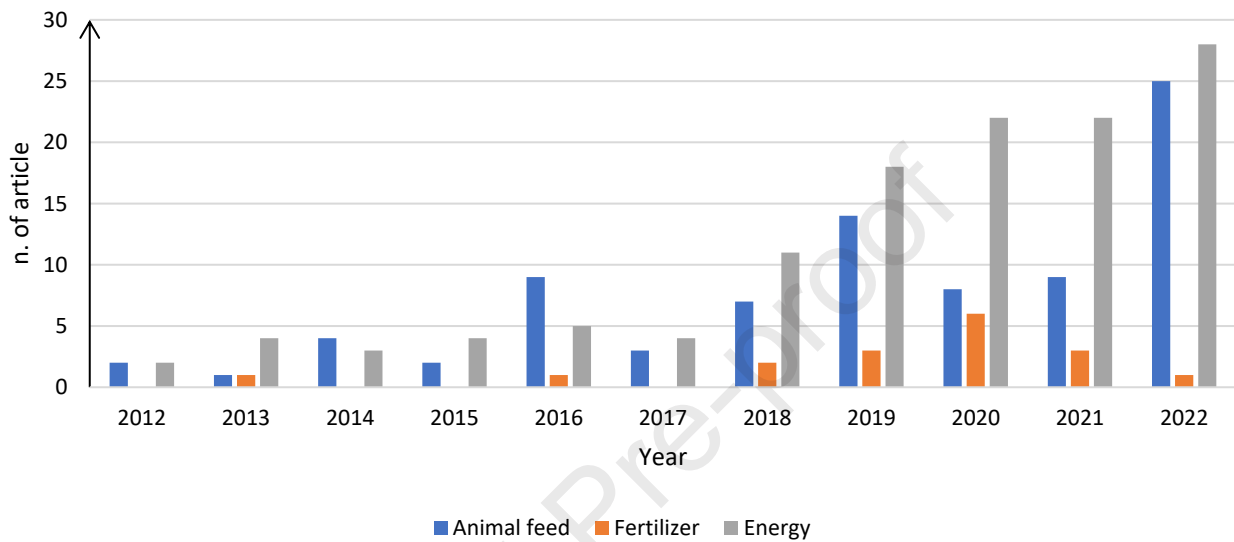
Sustainability in terms of the natural resource demands of protein-rich algal meal (versus soybean) for livestock feed applications	Microalgae, soybean	Pilot plant	n.s.	Protein	Microalgae and soybean for animal feed	the most exergetically inefficient processes are anaerobic digestion (66.47%), condensation (56.53% and 63.81%), inoculum production (54.98%) and drying (44.01%)	(Taelman et al., 2015)
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## 696 6 Temporal trends and future challenges of research

697 Research interest in the biorefinery of biomass from livestock has grown in recent years. Figure 9  
698 shows the biorefinery growth trends for three of the main bioproducts obtained.

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700

701 **Fig. 12.** Temporal trends of scientific articles

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703 The growth of interest is mainly due to biorefineries for bioenergy production. In the category  
704 “Energy”, anaerobic digestion and bioethanol production are the most widely used processes.  
705 However, a critical examination of the articles shows that this process is often conducted using  
706 traditional methods, as this is an established and widespread technology. In most cases, research  
707 focuses on process optimisation or evaluating matrices other than traditional matrices, which often  
708 use uncommon products. As shown in Figure 5, although the number of articles on anaerobic  
709 digestion has increased very abruptly over the past four years compared to the previous 7, this  
710 research contribution now appears to have reached a stage of stability. These considerations lead one  
711 to think that research in biorefineries will have to turn towards other forms of bioenergy, such as  
712 biofuels or upgrading systems, areas that exist but where there is still considerable scope for  
713 development.

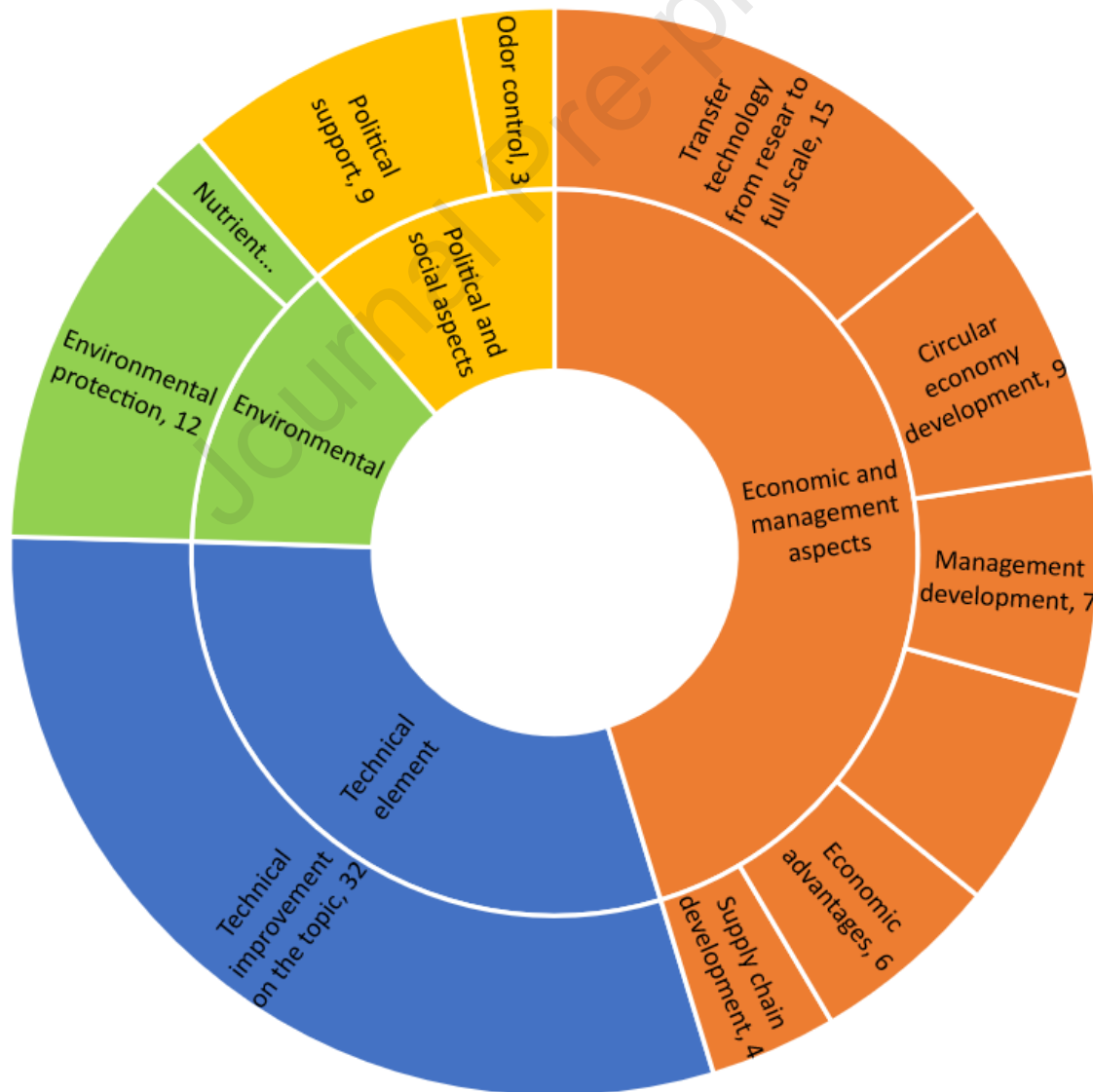
714 Nevertheless, in environmental sustainability, many authors have directed their efforts towards  
715 research dedicated to reducing the environmental impact and land consumption of livestock activities;  
716 in particular, many authors have demonstrated the importance of reducing the land consumption  
717 devoted to crops for animal feed production. Therefore, research into the production of ethanol,  
718 protein and other nutrients from agricultural byproducts and waste and from insect farming has  
719 gradually increased over the years. In particular, scientific contributions concerning insect breeding  
720 in biorefineries were very scarce until 2018 (only two registered articles) and were concentrated in  
721 the last four years, from 2019 to 2022 (18 articles).

722 The analysis of the articles made it possible to describe the areas of development of bioenergy, the  
723 objectives, and drivers for the development of these processes (Fig. 10a). Many authors recommend  
724 the development of methods and the improvement of technology; these objectives are particularly  
725 important regarding insect breeding, a relatively new field. Most scientific contributions recommend  
726 focusing on economic and management aspects. The transition from laboratory processes to full-scale  
727 plants requires testing technologies on progressively larger plants. For the complete application of  
728 biorefinery processes, it is necessary to undertake cost-cutting paths. Furthermore, exploiting the  
729 economic benefits derived from integrated resource utilisation approaches is necessary, a vision  
730 closely linked to the circular economy. Particular attention must be paid to the supply chain; many  
731 authors see the irregularity and seasonality of biomass as a possible point of weakness (and thus  
732 improvement) for the sector. Other authors identify environmental benefits as an essential driver for  
733 developing biorefineries. It is worth emphasising that, for many researchers, political support and the  
734 definition of rules and incentives are positive and, in some cases, necessary to spread these processes.  
735 Alongside the positive and developmental elements, the analysis of the articles identified obstacles  
736 and aspects of resistance to the spread of biorefineries (Fig. 10b). Biorefinery processes are still seen  
737 as very expensive, which hinders their spread on an industrial scale. This difficulty leads to a lack of  
738 reliable data on the application of these technologies in the real environment; much research is carried  
739 out in the laboratory or in pilot plants, which is why it is not easy to estimate the convenience and

740 impact of the same processes in industrial plants. The same applies to management practices, which  
 741 are still insufficient to guarantee full process reliability. Some authors see the lack of suitable politics  
 742 as a possible brake on the spread of biorefining. The population still views these technologies with  
 743 distrust, partly due to the lack of reliable regulations.

744 It should be noted that for many authors, some biorefinery processes can also undermine  
 745 environmental protection. Indeed, local nutrient accumulation problems arise as a result of biomass  
 746 exploitation. Furthermore, many processes are still significant energy and natural resource  
 747 consumers.

748



a)



b)

749 **Fig. 13.** (a) Possible areas of study, objectives, stimulating elements and (b) possible obstacles and elements  
 750 of resistance to the spread of biorefineries  
 751

752



## 753 **7 Conclusion**

754 This paper proposes an analysis of scientific articles on biorefinery processes applied in the livestock  
755 sector. Both processes that exploit biomass from livestock farming and processes that exploit biomass  
756 to produce livestock products were considered. A total of 293 articles published between 2012 and  
757 2022 were analysed. Most articles use manure as biomass, 123 articles, while the most considered  
758 product is bioenergy production, 123 articles. Finally, review articles, LCAs and technoeconomic  
759 articles were analysed to provide a comprehensive global view of the topic. Based on the achieved  
760 results, three key elements can be summarised:

- 761 i) Interest in the biorefinery of animal byproducts has steadily increased in recent years. The results  
762 confirmed the conclusions of previous studies; in fact, the most commonly used treatment was  
763 anaerobic digestion, with 84 articles. Research interest in this topic is steadily increasing;  
764 however, it is still linked to traditional processes and products, anaerobic digestion, and biogas.
- 765 ii) Currently, promising new areas of research are emerging. Concerning the biomasses used, new  
766 combinations between livestock manure and other biomasses, whether agricultural or  
767 civil/industrial, are being experimented with; in addition, insects, which can be an essential  
768 source of proteins and carbohydrates in all areas of biorefineries, are gaining attention. In terms  
769 of uses, biofuels are an area of significant research interest, an interest that is consistent with the  
770 policies of many institutions.
- 771 iii) Concerning the future direction of research, two scenarios can be imagined. If research is still  
772 autonomous in its choice of objectives, the use of livestock biomasses will probably continue to  
773 be applied to energy production and animal feed production; the cultivation of microalgae in  
774 liquid biomasses and the breeding of insects will likely gain importance. On the other hand, in  
775 the presence of a policy direction and, possibly, a system of incentives, the work of researchers  
776 and technicians may be directed more towards fields that are currently less explored, such as the  
777 production of bioproducts for the building industry or the manufacturing industry. In both cases,

778 innovations will certainly involve insect breeding, an up-and-coming sector in various  
779 applications: food production, animal feed, purification of wastewater, etc.

780 This analysis was limited to studying biorefineries in animal husbandry and did not devote as much  
781 attention to agriculture in general. The investigation could also be deepened by examining the  
782 different species of insects and other microorganisms involved in biorefining.

783 In conclusion, the results obtained confirmed and emphasised the role of biorefineries in livestock  
784 production systems in reducing the environmental impact of the agricultural system and in  
785 contributing to reducing the use of resources in other sectors.

786

### 787 **Acknowledgement**

788 This study was carried out within the Agritech National Research Center and received funding from  
789 the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA  
790 (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022,  
791 CN00000022). This manuscript reflects only the authors' views and opinions, neither the European  
792 Union nor the European Commission can be considered responsible for them  
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**Declaration of interests**

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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