Liquid organic fertilizers: Local knowledge to promote sustainable agriculture worldwide

Fertilizantes orgánicos líquidos: conocimiento local para promover la agricultura sostenible al nivel mundial

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ABSTRACT

Liquid organic fertilizers (LOF) or bioles are liquid bioinput products derived from the fermentation of vegetable and fruit wastes, manure, waste from the dairy industry, and others; their metabolic and microbiological composition varies according to the source of its raw material. Its biostimulant effect has been demonstrated on various crops of agronomic interest in some Latin American countries, such as Costa Rica, where it is produced in an artisanal way. To our knowledge, this is the first literature review of LOF from a technical perspective, showing the stages of their fermentation process, as well as factors that may affect it, and technological tools that will allow us to monitor their quality and safe application in crops. We found that the application of this traditional and local practice has great potential to be projected worldwide; however, more research is needed to standardize its composition and effects according to the type of crop. The use of biotechnological tools for making LOF would allow the determination of their metabolic and microbiological composition and, at the same time, the monitoring of the quality of the product, allowing its safe use in agriculture. Finally, the production and application of LOF within a sustainable agricultural model would favor a circular economy by using organic waste as a raw material, thereby reducing the cost of chemical product consumption.

Keywords: biol, agro-industrial waste, regenerative agriculture, biofertilizer, anaerobic digestion, soil fertility, agricultural productivity, sustainability.

RESUMEN

Los fertilizantes orgánicos líquidos (FOL) o bioles son unos bioinsumos líquidos producto de la fermentación de desechos vegetales y frutales, estiércol, desechos de la industria láctea y otros, cuya composición metabólica y microbiológica varía según la fuente de su materia prima. Se ha demostrado su efecto bioestimulante sobre diversos cultivos de interés agronómico en algunos países de América Latina, como en Costa Rica, donde este es elaborado de manera artesanal. Según nuestro conocimiento, esta es la primera ocasión en la que una revisión de literatura sobre FOL se aborda desde un punto de vista técnico, mostrando las etapas de su proceso de fermentación, así como factores que puedan afectarla y herramientas tecnológicas que nos permitirán monitorear su calidad y aplicación segura en cultivos. Encontramos que la aplicación de esta práctica tradicional y local tiene el gran potencial de ser proyectada a nivel mundial; sin embargo, requiere de más investigaciones para estandarizar su composición y efectos de acuerdo con el tipo de cultivo. El uso de herramientas biotecnológicas en la elaboración de FOL permitiría determinar su composición metabólica y microbiológica, y a su vez monitorear la calidad del producto permitiendo su uso seguro en la agricultura. Finalmente, la producción y aplicación de FOL dentro de un modelo de agricultura sostenible favorecería una economía circular al utilizar desechos orgánicos como materia prima, reduciendo el costo por el consumo de productos químicos.

Palabras clave: biol, residuo agroindustrial, agricultura regenerativa, biofertilizante, digestión anaeróbica, fertilidad del suelo, productividad agrícola, sustentabilidad.

Received for publication: July 7, 2025. Accepted for publication: October 7, 2025.

Doi: 10.15446/agron.colomb.v43n3.121362

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Introduction

The intensive use of fertilizers and agrochemicals increases agricultural productivity, but it generates global environmental problems such as greenhouse gas emissions, human health effects, and loss of biodiversity (Devi *et al*., 2022; Mitra *et al*., 2021). The consumption of fertilizers reached 195 million t in 2021. With the projected population growth of 9700 million by 2050, this dependence is intensifying. The consequent demand for food is expanding, increasing the need to optimize its use to guarantee food security (FAO, 2019).

The application of agrochemicals reduces soil fertility due to its degradation, heavy metal accumulation, and reduction in the number of beneficial microorganisms (Mandal *et al*., 2020). The filtration of nitrates and phosphates contaminates water sources, favoring eutrophication and compromising aquatic life (Hossain *et al*., 2022). The emission of nitrous oxide and ammonia contributes to climate change and air pollution (Ashitha *et al*., 2021). In addition, its inadequate management alters the balance of the soil, compromising agricultural productivity and sustainability (Rahman & Zhang, 2018).

Soil is essential for agriculture because it provides support, water, and nutrients to plants, and regulates the recycling of nutrients (Pahalvi *et al*., 2021). This dynamic ecosystem, where microbial biodiversity is indicative of health, is essential for agricultural productivity and pathogen suppression, ensuring water quality biodiversity and promoting food security (Glockow *et al*., 2024). Soil protection is vital, is necessary to support food security. This is possible through the implementation of sustainable agricultural practices by optimizing production without compromising the ecological balance through the application of bioinputs.

The use of bioinputs made from microorganisms, plant extracts, and plant residues increases soil fertility and nutrient uptake (Rocha *et al*., 2024). They are classified as biopesticides and biofertilizers; the latter include biostimulants such as microbial inoculants, protein hydrolysates, organic fertilizers, and seaweed extracts (Shahrajabian *et al*., 2021). Among these are liquid organic fertilizers (LOF) or bioles, which are bioferments obtained by anaerobic digestion of agroindustrial residues through microbial consortia, similar to the biogas production process from agricultural waste, where the liquid effluent called bioslurry has proven to be effective for agricultural fertilization (Bonten *et al*., 2014; Gil Ramírez *et al*., 2023).

In Latin America, LOF represent a traditional agricultural practice developed empirically, without formal scientific support or defined chemical composition, but with significant results in the development of crops (Jara-Samaniego *et al*., 2021). They are produced on small farms using low-cost anaerobic fermenters, representing an accessible solution for rural communities and allowing the integration of traditional knowledge with sustainable practices (Aguado *et al*., 2023; Sánchez-Ramón *et al*., 2024).

This local technology improves traditional agricultural practices by increasing soil fertility and the production of crops with less environmental impact, laying the foundations for modern sustainable agriculture (Sánchez-Ramón *et al*., 2024). It is presented as an effective tool of regenerative agriculture to address the global challenges of food security and the conservation of natural resources. However, its preparation and application in crops are limited to regional practices. Farmers lack access to technical-scientific information and technology to support the effectiveness of their products, quality standards, and safe application in crops. This is due to the lack of adequate advice and the difficulty in transferring this information and technology from academia to the field, as well as the lack of funding for research and development, where farmers and academia can join forces to develop joint research (Bullor *et al*., 2023). In this context, the aim of this review was to describe how these LOF are produced and highlight their potential in promoting sustainable agricultural practices at a global level, revealing and promoting their positive impact on the transition towards production models that are more responsible and resilient, and allowing us to reduce the negative impact generated by the excessive use of agrochemicals. In addition, we aimed to demonstrate how the use of technological tools and science can help us to understand the production process, as well as guarantee its quality, beneficial effects on plants, and its safe application on crops.

LOF composition

LOF are biostimulants produced by anaerobic digestion of organic waste through a consortium of microorganisms present in the raw material used in their production. Its sustainable production converts agricultural byproducts into valuable agrarian products that adapt local resources such as crop residues, manure, whey, mountain microorganisms, molasses, and minerals (Fig. 1). This variability generates multiple formulations that share basic principles but differ in composition (Gallegos *et al*., 2022).

Animal manure (bovine, pig, avian, or goat) is the most commonly used input, recycling livestock byproducts and reducing the accumulation of waste (García Narváez, 2018). It provides organic matter that improves water retention and soil structure, as well as increases the contents of nutrients that stimulate microbial activity and facilitate the transformation of organic compounds into assimilable forms for plants (Prasad *et al.,* 2017). Its composition varies according to the animal's diet, influencing its effectiveness. In addition, its use requires adequate management to avoid gas emissions, accumulation of salts, and the presence of pathogens (García Narváez, 2018).

Plant residues maintain the C/N ratio due to their contribution of carbon, allowing the efficient decomposition of organic matter and the progressive release of nutrients (Vélez Quijije & Zambrano Solórzano, 2019). Whey from the dairy industry promotes the proliferation of lactic acid bacteria that ferment organic matter and stabilize the LOF, improving their effectiveness as biostimulants (Sirmacekic *et al.,* 2022). Molasses provides fermentable sugars that stimulate microbial activity and essential minerals (K, Ca, and Mg) that improve soil fertility. However, their use must be regulated to avoid alterations in the osmotic balance (Stephen *et al*., 2024).

Mountain microorganisms (MM), microbial communities of forest soils rich in organic matter, are used as inoculum because of their ability to degrade organic matter and fix nutrients, and also function as a type of biofertilizer (Umaña Carmona, 2017). These bacteria include phototrophic and lactic acid bacteria, fungi, yeasts, actinomycetes, phosphorus solubilizers, and nitrogen fixers (Castro Barquero *et al*., 2015). They improve the structure and fertility of the soil by increasing the availability of N, P, and K. However, their survival during fermentation depends on the composition of the soil, temperature, and humidity (Umaña *et al.,* 2017).

Flexibility waste selection allows the use of agricultural byproducts to be optimized, reducing the generation of waste and promoting a circular economy. The list of inputs will vary according to its location, available resources, and desired objective. The LOF denomination will depend on this (Tab. 1).

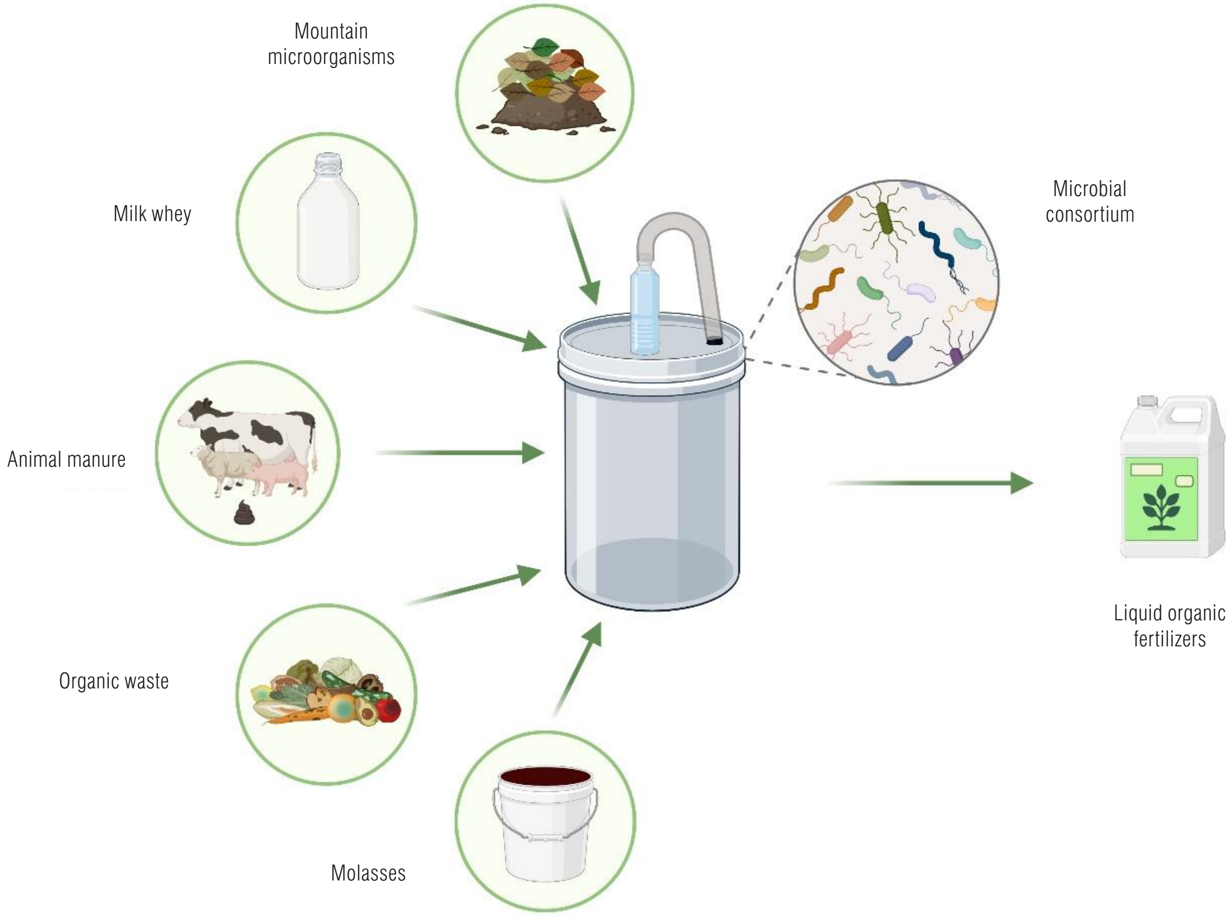

FIGURE 1. Schematic representation of the artisanal LOF production process using different raw materials.

TABLE 1. Denomination of LOF based on their preparation using different raw materials.

Denomination	Composition	Crop	Reference
Bovine LOF	Bovine manure	*Hordeum vulgare* L.	Tambo Laime *et al.* (2016)
Poultry LOF	Chicken manure	*Capsicum annuum* L.	Medranda Vera *et al.* (2016)
LOF for plant nutrition (Bfao)	Goat manure, wood ash, bentonite, eggshell, cow's milk, and bone ash	*Lactuca sativa*	Funes-Pinter *et al.* (2022)
Mountain microorganisms (MM)	Forest mulch	*Brassica rapa* subp. *pekinensis* var. Taranko F1	Castro-Barquero *et al.* (2020)
Biofertilizer of cocoa harvest residues	Beef manure, yeast, sugar, cocoa crop residues, chicken manure, rock phosphate, and dolomite	*Theobroma cacao* L.	Lucano-López and Alegre-Orihuela (2023)
Fruity LOF	Residues of papaya, mango, banana, alfalfa, molasses, whey, and activated microorganisms	*Amaranthus caudatus* var. Oscar Blanco	Tejada Vizcarra *et al.* (2023)
Foliar LOF	Bovine manure, molasses, and *Mucuna* sp.	*Heliconia psittacorum* cv. Tropica	Linares-Gabriel *et al.* (2017)
LOF for soil	Bovine manure, molasses, and soybean meal	*Heliconia psittacorum* cv. Tropica	Linares-Gabriel *et al.* (2017)

Fermentation process of LOF

The production of the LOF is carried out in a handmade closed fermentation system. It consists of a 20-300 L plastic tank with a hermetic lid where raw materials are added at 80% capacity and mixed with water. Once the temperature of the mixture is equal to the outside temperature, the fermentation system is closed. The lid is connected, by means of a hose, to a bottle with water to allow the escape of gases, and the system is allowed to ferment under shade for weeks to months (Gallegos *et al*., 2022; Tejada Vizcarra *et al.,* 2023). Previously, it was reported that at one-week, microbial activity is dominated by bacteria; at two weeks by fungi and actinomycetes; and at three weeks, actinomycetes show greater activity (Umaña *et al.,* 2017).

During fermentation, organic matter degrades anaerobically, converting organic carbon to CO_2 and CH_4 through oxidation-reduction reactions. A microbial consortium orchestrates this complex and delicate process through four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Adekunle & Okolie, 2015) (Fig. 2).

In hydrolysis, carbohydrates, lipids, and proteins are converted into sugars, long-chain fatty acids, and amino acids, respectively, mainly because of the activity of anaerobic bacteria such as *Bacteroides*, *Clostridium,* and *Bifidobacterium* and some facultative bacteria such as *Streptococci* and *Enterobacteriaceae* (Weiland, 2010). During acidogenesis, the hydrolysis products are converted into volatile fatty acids (VFAs), organic acids, H_2, CO_2, and NH_3, with the involvement of the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Lim *et al.,* 2020). During acetogenesis, VFAs are transformed to acetate, CO_2, and H_2 via the activity of bacteria such as *Acetobacterium woodii* and *Clostridium aceticum* (Adekunle & Okolie, 2015; Weiland, 2010).

The hydrolysis products, acetate, CO_2, and H_2 transform to CH_4 via two routes: the acetotrophic route, which produces 65-70% of the methane via acetate through bacteria such as *Methanosaeta harundinacea* and *M. concilii*, and the hydrogenotrophic route, which uses CO_2 and H_2 through species of the genera *Methanobacterium* and *Methanospirillum*. Methanogenic bacteria are strictly anaerobic and very vulnerable to low concentrations of O_2, which is one of the most critical stages of this process (Cazier *et al.,* 2015; Gerardi, 2003). Once anaerobic digestion is finished, the system is opened, and the content is filtered to separate the liquid fraction (LOF) from the solids. LOF are stored in hermetic containers under shade and in a cool place until use (Rojas-Pérez *et al.,* 2020).

Factors affecting the fermentation of LOF

Factors influencing the characteristics of liquid mountain microorganisms (MM), such as LOF, have been previously evaluated. In terms of the MM origin, the pH and concentrations of yeasts, bacteria, nitrogen fixers, and phosphorus solubilizers can fluctuate. When forced air is applied to the MM, the substrates are quickly depleted, and the pH increases. In anaerobiosis without a hermetic lid, the pH rises slowly, lactobacilli and yeasts are more permanent, and in airtight anaerobiosis, microbial growth is negatively affected. When the inoculum by half the microbial population increases, the excess of molasses can affect the activation by increasing the electrical conductivity in the MM-type LOF. The enrichment of these LOF with salts, such as K, can increase the microbial concentration (Castro-Barquero & González-Acuña, 2021).

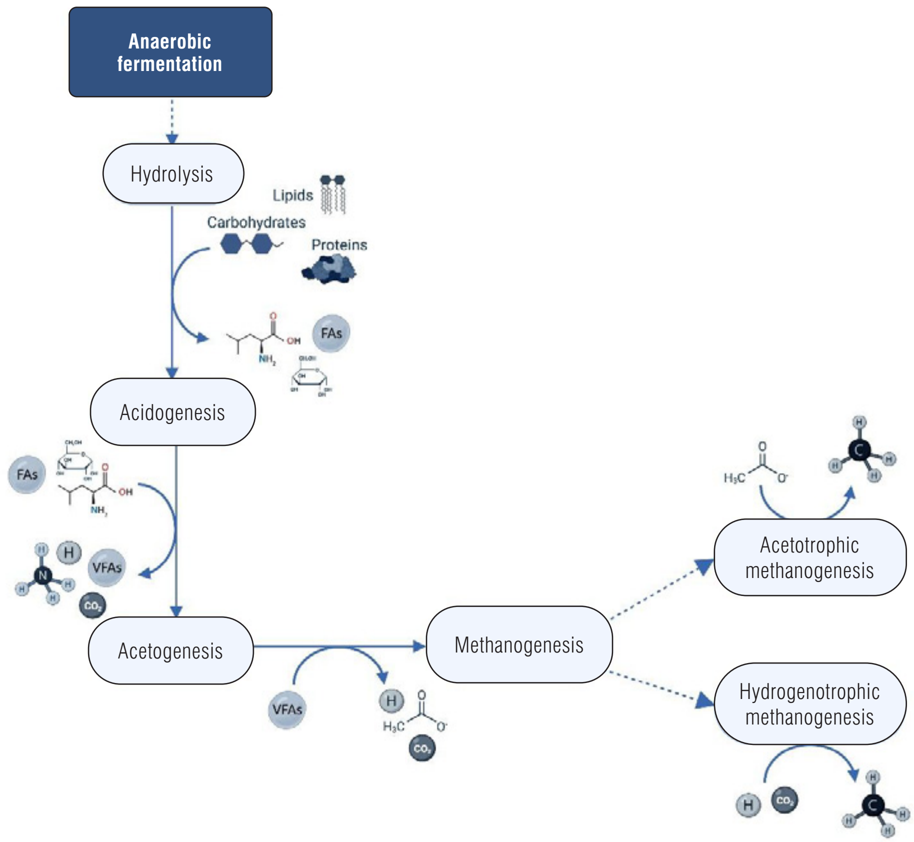

FIGURE 2. Essential steps carried out during the anaerobic fermentation process of organic matter for LOF production.

Temperature: In anaerobic fermenters, microorganisms are sensitive to thermal fluctuations, which affect the degradation of organic matter (Angelidaki *et al.,* 2003; Mao *et al.,* 2015). Methanogenic bacteria are active at 30-35°C (mesophilic) and 50-60°C (thermophilic) and are inhibited between 40-50°C. In contrast, hydrolytic bacteria are less sensitive to thermal changes than acetolytic and methanogenic bacteria (Gerardi, 2003). An increase of 10°C can harm methanogenic microorganisms but favor acidogenic organisms. However, this can increase free ammonia by inhibiting microbial activity (Gerardi, 2003). However, a thermal increase can eliminate pathogenic bacteria, ensuring the safety of the LOF (Bendixen, 1994).

pH: pH affects anaerobic digestion and its products by influencing enzymatic activity (Mao *et al.,* 2015; Wainaina *et al.,* 2019). Initially, it decreases due to the synthesis of volatile acids and increases when these acids are consumed. Ammonia accumulates during protein degradation, although its stability is affected by the accumulation of CO_2 (Kondusamy & Kalamdhad, 2014; Weiland, 2010). Hydrolytic bacteria are favored at pH 6-7, whereas acidogenic microorganisms are stimulated at pH 5.5-6.5 (Jiang *et al.,* 2013; Mao *et al.,* 2015). Methane production is optimal at pH 7-8, and it is stopped below 6 or above 8.5 (Weiland, 2010). In sheep manure LOF, pH values of 3.5-4.0 have been recorded, whereas in bovine LOF, pH values of 6.9-7.8 have been reported (Gil Ramírez *et al.,* 2023; Pomboza-Tamaquiza *et al.,* 2016).

Raw material: In anaerobic digestion, the conversion of inorganic nutrients from acetate to methane is limited. The macronutrients N and P and mineral nutrients Co, Fe, Ni, and S are unique to these bacteria because of their enzymatic activity (Gerardi, 2003). The residues of fruits, vegetables, molasses, whey, and animal excreta are ideal for the production of LOF (Jara-Samaniego *et al.,* 2021). Their nutritional effect on microbial activity depends on its availability as a soluble or solid fraction, whether it is hydrolyzable or not, and the origin of the inputs used (Gil *et al.,* 2019). LOF produced from fermented grass have greater contents of N, P, Mg, S, Fe, Zn, Mn, and B, and those made from manure can present higher levels of Ca and Cu. Likewise, the use of finch leachate tends to reduce the number of yeasts by increasing the pH of the LOF (Araya Alpizar, 2010).

C/N ratio: The C/N ratio determines the efficiency of the process, and a correct balance favors microbial activity and the degradation of organic matter (Kondusamy & Kalamdhad, 2014). Compared with animal manure, plant residues present higher C/N ratios (Ngan *et al.*, 2020). The optimal C/N ratio for anaerobic fermentation is 25:1. High ratios inhibit growth due to a lack of N. In contrast, low ratios cause ammonium accumulation and inhibition due to toxicity (Mao *et al.*, 2015).

Retention time: The retention time is the time required to completely degrade the organic matter. It is related to the rate of microbial growth, which affects the efficiency of anaerobic digestion (Mao *et al.*, 2015). It is defined by the complexity of the substrate and added load, which determines organic hydrolysis (Wainaina *et al.*, 2019). In LOF, the fermentation time depends on the environmental conditions, since high temperatures shorten the fermentation time (summer days). In contrast, low temperatures increase fermentation time (Soria Fregoso *et al.*, 2001).

How do we determine the quality of LOF?

Farmers consider that the LOF are of good quality when they are dark brown or olive-green in color and no longer have a fetid odor, indicating the complete degradation of organic matter (Devarenjan *et al.*, 2019; Rojas-Pérez *et al.*, 2020). During and at the end of fermentation, it is necessary to monitor the pH, temperature, and other parameters to ensure the LOF are suitable for their application in crops. There are no reference values for determining the quality of the LOF since it depends on the composition of the raw material. Consequently, the nutritional and microbial composition depends on its formulation (Jara-Samaniego *et al.*, 2021; Rojas-Pérez *et al.*, 2020).

Microbiological parameters: The beneficial qualities of the LOF are attributed to their microbial community, whose diversity depends on the fermentation conditions and raw material (Sundberg *et al.*, 2013). Plant growth-promoting bacteria (PGPB) favor the absorption of nutrients in plants, synthesizing growth regulators or protecting plants against pathogens (Glick, 1995). They are divided into functional groups: nitrogen fixers (FNs), phosphorus solubilizers (SPs), lactobacilli (Lac), and yeasts (Lev) (Castro-Barquero & González-Acuña, 2021).

Previously, strains of lactic acid bacteria with potential agricultural use have been isolated from LOF because of their ability to produce indoleacetic acid (IAA). In the presence of L-Trp, *Lactiplantibacillus plantarum* and *L. carotarum* produce high concentrations of IAA, whereas *Lacticaseibacillus paracasei* has a more variable response (Montero-Castro *et al.*, 2024). Other studies have described specific microorganisms in different stages of fermentation (Castro-Barquero *et al.*, 2020), enabling standardization of the production of LOF and guaranteeing their biostimulant consistency. It is important to detect coliforms as indicators of fermentation. However, the absence of fecal coliforms has been reported in some LOF, allowing their safe application (Gil Ramírez *et al.*, 2022; Jara-Samaniego *et al.*, 2021).

Physicochemical parameters: The salt content determines the electrical conductivity of the LOF. LOF with low conductivity are ideal for organic agriculture. In contrast, those with high conductivity can be applied in crops resistant to salinity (Jara-Samaniego *et al.*, 2021). Likewise, the quality of the LOF depends on their concentration of macro- and micronutrients, which vary according to the raw material (Gallegos *et al.*, 2022). Although some metals are micronutrients, their high concentrations can cause toxicity (Mendoza *et al.*, 2017). They usually come from livestock excreta and plant residues and are essential for monitoring their concentrations (Yasar *et al.*, 2017). However, in a few studies, they have been quantified in LOF.

Macro- and micronutrients are necessary for the correct development of crops. LOF are a rich source of nutrients with concentrations varying according to the raw material used (Tab. 2).

TABLE 2. Concentration of macro- and micronutrients reported in different types of LOF.

Nutrient	Concentration	Reference
N	290-690 mg L^{-1}	Gil Ramírez *et al.* (2022); Suárez Segura (2009)
P	17.2-22 mg kg^{-1}	Funes-Pinter *et al.* (2022); Gil Ramírez *et al.* (2023); Soria Fregoso *et al.* (2001)
K	111.7-7574.3 mg L^{-1}	Gil Ramírez *et al.* (2023); Rojas-Espinoza *et al.* (2023); Soria Fregoso *et al.* (2001)
Mg	59.3-153.2 mg L^{-1}	Gil Ramírez *et al.* (2023); Soria Fregoso *et al.* (2001)
Ca	11.3-978 mg L^{-1}	Aguado *et al.* (2023); Zagoya Martínez *et al.* (2015)
S	0.360-341 mg L^{-1}	Gil Ramírez *et al.* (2023); Suárez Segura (2009)
Fe	1.53–9.33 mg L^{-1}	Gil Ramírez *et al.* (2023); Rojas-Espinoza *et al.* (2023); Soria Fregoso *et al.* (2001)
Cu	0.080-0.225 mg L^{-1}	Gil Ramírez *et al.* (2023); Soria Fregoso *et al.* (2001)
B	16.1 mg L^{-1}	Suárez Segura (2009)
Mn	0.001-71.80 mg L^{-1}	Aguado *et al.* (2023); Quiñones Ramírez *et al.* (2016)
Zn	0.003-1.33 mg L^{-1}	Aguado *et al.* (2023); Rojas-Espinoza *et al.* (2023)

Biostimulant effects of LOF in several crops

The LOF can be applied to both leaves and soil, alone or mixed with fertilizers, to favor plant growth (Linares-Gabriel *et al.,* 2017; Monge-Pérez *et al.,* 2022). The dilution of LOF in water optimizes their application, ensuring the availability of nutrients and avoiding the accumulation of salts (García Narváez, 2018). The standard dilution suggested in studies is 1:20 (LOF:water), as it provides a balance between nutrient availability and safety for crops (García Narváez, 2018; Xiu Canche, 2018).

In corn, LOF concentrations of 30% generate higher productivity than 20%, as well as adding lactic acid bacteria to LOF at 30% promotes a greater height in plants; meanwhile, some reports also show beneficial effects on the crop when 60% LOF are applied (García-Gonzalez *et al*., 2020; Vélez Quijije & Zambrano Solórzano, 2019). In turn, it has been reported that LOF based on plant residues and MM at a 100% concentration improve the biological and chemical indices of the soil (Umaña *et al.,* 2017). In addition, the application of biocontrol agents in soil increases the population of beneficial fungi such as *Trichoderma* sp. It reduces pathogens such as *Penicillium* sp. and *Fusarium* sp. (Monge-Pérez *et al.,* 2022). Various studies have shown that LOF benefit plant production, yield, and health by acting as biostimulants since their microbial and metabolic composition influences plant growth and development.

- Sweet pepper (*Capsicum annuum*): Medranda Vera *et al*. (2016) reported that the application of LOF based on chicken manure resulted in yields of 34.7 mg ha^{-1} with respect to the weight of the fruits and 32.2 mg ha^{-1} when bovine LOF was applied, both of which exceeded the yield of 17.9 mg ha^{-1} per area of application. The growth and development of pepper fruits increased by 48% when chicken manure was used, and 44% when bovine LOF was used. This is because chicken manure has a relatively high nutritional content. In contrast, bovine manure has a relatively low concentration of minerals, since the mineralization rate is approximately 35% in cattle and 90% in poultry in one year. In this study, a greater height, number of plants, and diameter, length, and weight of fruits were observed, which was more notable when poultry manure was applied.
- Beans (*Phaseolus vulgaris*): The application of LOF enriched with diammonium phosphate (DAP) and urea resulted in greater productivity, generating more pods, greater weight of the seeds, and yields of 3,284 kg ha^{-1} compared with a control whose yield was 3,006 kg ha^{-1} (Santin Chávez, 2017). In addition, the application of bovine LOF and coffee husks has increased the average height of bean plants to 16.69 cm (Escalante, 2023). On the other hand, Morales García and Tuarez Bravo (2023) reported that the foliar application of LOF based on 10% banana rachis in beans resulted in a yield of 218.50 kg ha^{-1}. In contrast, their edaphic application increased the diameter of the stem by promoting the absorption of minerals and improving the microbial activity of the soil.
- Potato (*Solanum tuberosum*): In potato crops, Condori-Mamani *et al*. (2017) reported that when LOF based on alfalfa and eggshell at concentrations of 1:1 and 1:3 were used, maximum heights between 33.8 cm and 40.4 cm were observed for the accessions Alq'a pali, Polo, Polônia, and Amajaya, whereas for Canatilla, Janq'o Shock, Imilla Blanca, Ajahuiri, Saq'ampara, Q'aysalla, Sani imilla, and Layam Q'aysa, heights between 23.4 cm and 29 cm were reported. A significant difference was detected in the foliar development of the Alq'a pali, Amajaya, and Laram Q'aysalla accessions, which was attributed to the concentration of LOF applied and their phytohormone content. Likewise, a significant impact on crop yield was observed, reaching averages of up to 17.76 t ha^{-1}.
- Spinach (*Spinacia oleracea*): In spinach, Siura *et al*. (2009) evaluated the biofertilizing effects of various types of LOF. They reported that at concentrations of 20% and 100%, there was an increase in production, reaching yields of up to 25.80 t ha^{-1} and significantly improving the average natural yield of 14.9 t ha^{-1} per year. In addition, no effects were observed on the external quality of the spinach since the measurements of height, length, and width of the leaf blade remained constant. These results demonstrate the effectiveness of the LOF in improving the productivity of spinach without compromising its quality, indicating that the LOF are a viable alternative in sustainable agriculture.
- Lettuce (*Lactuca sativa*): In a lettuce crop, Pomboza-Tamaquiza *et al*. (2016) reported that the application of LOF at 6% improved its final productivity, which was 183 kg/plot compared to a control treatment, with a yield of 72 kg/plot. In addition, it promoted a greater diameter of heads (25.9 cm) and a greater commercial weight (1.14 kg), stimulated the growth of lettuce, and reduced its harvest time by 10 d by improving the efficiency of its vegetative cycle and contributing to its healthy development. Similarly, other key agronomic variables, such as the height of the plant, the diameter of the stem, and the weight of the product, were affected by the application of the LOF.
- Cacao (*Theobroma cacao*): In their study, Lucano-López and Alegre-Orihuela (2023) evaluated a type of LOF with

high nutritional content made with cocoa residues, manure, yeast, sugar, chicken manure, and phosphate rock in cocoa plantations. During its application in the dry season, an increase in the weight, diameter and yield of the fruits was observed, as well as an improvement in the physical characteristics of the cocoa beans when the LOF was applied at 10% and 15%, and a greater production of fruits when the LOF was used at 15%, indicating that LOF generate greater resistance and productivity in cocoa plants under drought conditions. On the other hand, the application for short periods in the rainy season did not significantly differ, suggesting that its efficiency is linked to continuous and prolonged application.

- Tomato (*Solanum lycopersicum*): In tomato, Aguado *et al*. (2023) reported that by applying 5% LOF to tomato seeds, germination is reduced, whereas in greenhouses, the application of 5%, 10% and 15% LOF has positive nutritional effects, although the effects are less than those of commercial fertilizers. Additionally, Castro Barquero *et al*. (2015) evaluated a soybean–tomato rotation system in a greenhouse for 24 months with individual and combined inoculations of *Azospirillum oryzae*, *Pseudomonas fluorescens*, *Bacillus subtilis,* and MM. In general, the application of microorganisms favored the microbial activity of the soil. Its application in mixtures increased the contents of C and N, the pH, electrical conductivity, and concentration of bacteria and actinomycetes. On the other hand, the application of MM increased the concentration of fungi and the biomass of the crop, in addition to the contents of macro- and micronutrients in tomato leaves, showing positive effects and being validated as an agricultural input.
- Mustard (*Brassica rapa* subsp. pekinensis): In mustard crops, Castro-Barquero *et al*. (2020) reported that when MM is applied, there is a reduction in the severity of *Plasmodiophora brassicae*, a pathogen that causes cruciferous herniation, which decreases yield by 50-60% and generates economic losses. In their study, the MM, which acts as a pathogen-suppressing agent, demonstrated potential as a sustainable biological control agent. Their microbial diversity contributes to the protection of crops, improves the health of the soil, and reduces dependence on the use of agrochemicals. These results highlight the importance of exploring its efficacy in different agricultural systems to optimize its use when combating pest attacks.
- Alfalfa (*Medicago sativa*): Previously, Gil Ramírez *et al*. (2022) evaluated the effects of different concentrations of cow manure-based biol on the growth and development of alfalfa. In general, when the LOF were applied, the dry weight of the plants increased over time. The application of LOF at 25%, 50% and 100% favored the development of the plants, and with a concentration of 50% it allowed a greater number of leaves and a greater size in the plants. The authors concluded that both the growth and development of alfalfa plants are stimulated by the application of LOF, especially at a concentration of 50%.
- Rice (*Oryza sativa*): In the rice crop, Diaz Almea and Contreras-Miranda (2022) tested different concentrations of LOF based on animal manure, showing variable effects compared with other treatments. When LOF at 25 % were applied, the plants exhibited a greater height and a greater number of panicles; meanwhile, LOF at 10% provided a longer panicle length. Nevertheless, the application of humic acids at 10% resulted in a greater number of rice tillers, and a higher productivity was observed when the chemical treatment was applied (Diaz Almea & Contreras-Miranda, 2022).

Can LOF help improve soil health?

LOF and organic fertilizers generally favor the health of soil by increasing its fertility and improving its structure and composition, both chemical and microbial, and its content of organic matter (Ang *et al*., 2023; Hossain *et al*., 2018). They help to increase the water retention capacity in the soil, improve the availability of nutrients, promote the exchange of cations, and reduce their erosion (Hossain *et al*., 2018; Lolamo *et al*., 2023; Monge Pérez *et al*., 2022).

In a previous study, Monge-Pérez *et al*. (2022) reported that the application of Fertibiol 45 L® biofertilizer improved the characteristics of a soil used for the production of pitahaya; the soil was clayey, with low acidity, and high macro- and micronutrient contents, but with a low concentration of P. The application of the LOF significantly increased the concentration of nutrients in the soil, primarily by increasing the availability of P (+ 112%), S (+ 1200%), and Zn (+ 18%), in addition to increasing the C/N. It increased the population of beneficial fungi, such as *Trichoderma* sp. It suppressed the presence of pathogenic fungi such as *Penicillium* sp. and *Fusarium* sp. in the soil. This study revealed that the application of LOF can both increase the availability of nutrients in the soil and restructure the microbial community by promoting the presence of beneficial microorganisms and reducing the abundance of pathogens, suggesting that LOF can be used as alternatives for the biological control of pests.

The presence of compounds in the LOF, such as organic acids malate, citrate, and oxalate produced by some microorganisms, could favor the mobilization and solubility of nutrients such as Zn, K, and P, regulate the soil pH, and promote enzymatic activity (Ammar *et al*., 2023; Macias-Benitez *et al*., 2020). In addition to improving the soil microbiota and strengthening the immune system of plants, the resistance of LOF to pathogens can increase. Some LOF include plant extracts with insecticidal or antifungal properties, which enhance their protective effect. In general terms, their use could promote ecological balance in agroecosystems, promoting the presence of beneficial organisms in the soil that naturally regulate pest populations and improving their fertility, especially in soils that have been severely damaged by the application of agrochemicals.

LOF in the circular economy

LOF are key for sustainable agriculture because they promote a circular economy through the use of waste and the reduction of costs due to chemical fertilization (León Torres *et al.,* 2019). Their diverse microbiological composition allows for the protection of crops, reducing the dependence on the use of pesticides (Manzano *et al.,* 2023). Future research should focus on quantifying the production costs of LOF in comparison with the application of agrochemicals. It is necessary to evaluate the direct savings and indirect benefits of their application, such as soil regeneration and emission reduction (Xie *et al.,* 2022).

Previous studies indicate that producing LOF is less expensive, with prices of US$ 0.03-0.09 per liter for LOF based on cow manure and US$ 0.08-0.24 per liter for LOF from fermented grass (Araya Alpizar, 2010). The benefit-cost analysis reveals returns of up to US$ 1.35 per dollar invested, which represents a viable alternative in sustainable production systems (León Torres *et al.,* 2019). Studies on bioslurry increase the understanding of its economic impact, where savings of up to US$ 1,386 per hectare have been reported for its application in rice and wheat (Kumar *et al*., 2023); therefore, integrating these aspects would optimize the implementation and integration of LOF in agricultural production.

It is essential to develop standardized methodologies to evaluate the environmental impact of LOF, primarily the reduction in methane and nitrous oxide emissions derived from the management of organic waste, whose improvement has already been reported by the application of bioslurries (Zhao *et al.,* 2024). The transition toward the use of LOF as part of a circular economy reduces costs, improves environmental sustainability, and promotes a regenerative approach that contributes to the food security and well-being of agricultural producers, which is a practice with great potential for its worldwide application.

Future prospects

Although the LOF production has traditionally been an artisan practice based on empirical knowledge, technological tools as biotechnology and others offers advanced tools to optimize this process, transforming it into a practice based on technical-scientific criteria that allow key aspects such as optimizing its composition, ensuring its quality, improving its effectiveness and guaranteeing its safety to be addressed (Singh *et al*., 2024).

The use of molecular techniques, such as polymerase chain reaction (PCR) and quantitative PCR (qPCR), will allow the rapid and precise detection of pathogens such as *Salmonella* spp. and *Escherichia coli* in LOF (Paraguison-Alili *et al*., 2021). To guarantee the quality of the LOF, traditional and modern microbiological techniques are also used. These were, for example, the use of selective media for the isolation of pathogens, the measurement of total coliforms as indicators of contamination, and biochemical tests that confirm the identity of microorganisms, as well as the count of colony-forming units (CFUs) (Kim & Kim, 2021).

On the other hand, optical and electronic microscopy can allow detailed visualization and characterization of the microorganisms present in the LOF, identifying the morphology and structure of microorganisms, which helps to differentiate between beneficial and pathogenic species (Palladino *et al*., 2024). The use of high-resolution microscopy would aid in understanding the interactions of the microorganisms present in the LOF with the microorganisms present in the soil and with the crops. Through scanning electron microscopy (SEM), the rhizosphere of plants can be spatially located to identify the organisms responsible for the cycling of carbon and other nutrients, and to study their effects on plants (Liu *et al*., 2021). Similarly, transmission electron microscopy (TEM) and fluorescence microscopy have allowed the study of microbial interactions in soil microstructures, which could be applied to study these interactions in soils treated with LOF (Li *et al*., 2004; Watteau & Villemin, 2018).

Notably, these quality tests must be carried out not only on the final product but also throughout the anaerobic digestion process. This ensures that the process is carried out efficiently and without creating conditions that favor the growth of pathogens or the loss of nutrients. In addition, enriching LOF with specific beneficial microorganisms, such as nitrogen-fixing bacteria, phosphorus solubilizers, and arbuscular mycorrhizal fungi, not only improves the fertilizing properties of the LOF but also generates competition with pathogens, strengthening the suppression of diseases in the soil (Bhardwaj *et al*., 2014).

Controlled fermentation is another key aspect of the production of high-quality LOF. Constant monitoring of parameters such as pH and temperature through specialized sensors guarantees an optimal environment that favors the activity of beneficial microorganisms and inhibits the growth of pathogens. The use of automated bioreactors provides a sterile and controlled environment during the production of the LOF, minimizing the risks of contamination. These technological innovations have increased the quality standards of biodiesel, ensuring that it is safe and effective for use in agriculture (Palladino *et al*., 2024).

In recent years, metagenomics has emerged in response to the rapid and efficient identification of the entire microbial community present in a sample, overcoming the limitations that microbiological culture techniques have presented for microbial identification (Lim *et al*., 2020). Through the sequencing of 16S rRNA, 18S rRNA, and ITS regions, metagenomics will allow us to explore the structure of the microbial population during the anaerobic digestion process and to understand how it changes with respect to the fermenter's operating conditions (Lema *et al*., 2023).

Several studies include analyses of microbial composition in anaerobic digesters for the production of biogas, reporting a significant number of bacteria like *Actinobacteria*, *Proteobacteria*, and *Euryarchaeota* (Sundberg *et al*., 2013). However, metagenomics can be applied to the study and monitoring of the biodegradation process so that we can understand how the dynamics within the anaerobic digester change throughout it and, in turn, the microbial composition present in the final product.

Similarly, by using metagenomics, we could understand and address the nutritional needs of crops in a more specific way, understand the interactions between these microbial communities and crops, and characterize different formulations of LOF that could be applied according to the type of cultivation and its stage of development.

Through the assembly of reads (long or short) of metagenomics libraries and the implementation of various algorithms and bioinformatics tools, we can identify the mechanisms of plant–-microorganism interactions, such as the synthesis of enzymes, the production of siderophores and other biostimulants activated as a biotic and abiotic stress response, and processes such as N fixation, the solubilization of P, and the translocation of other nutrients (Chukwuneme *et al*., 2021; Xiao *et al*., 2024).

On the other hand, the LOF could have a diverse composition of metabolites that have not yet been described. Its identification and study by applying metabolomics would allow us to understand its dynamics and changes during its production process (Alawiye & Babalola, 2021). Likewise, it would allow us to characterize this bioinput, obtain a complex database of metabolites that are part of its composition and that have a biostimulant effect in crops, understand the mechanism of interaction of these metabolites with plants, and study the functions of the microbial community of the LOF at the molecular level (Manickam *et al*., 2023).

Together, these technological tools could allow the safe use of LOF for their application in crops, significantly reducing the risks to human health and the environment, and transforming the artisanal practice into a process with a technical and scientific foundation. Science plays a fundamental role in this process by providing the necessary technical and methodological bases to guarantee the quality, safety, and efficacy of this bioinput. However, it is necessary to ensure that farmers have this technology. The support from governmental authorities is essential, as it provides funds for the development of research projects, where producers and research centers can be involved, allowing not only the technology interchange but also the exchange of information.

In addition, these innovations are expected to contribute to agricultural sustainability by reducing dependence on chemical inputs and promoting more environmentally friendly practices. However, it will be necessary to deepen the research and standardization of these processes to achieve wide and practical implementation in modern agricultural systems as well.

Conclusions

In this review, we provide detailed information about the reported effects of LOF on different crops of economic importance. In the same way, this is the first time a review addresses this subject in a more technical manner, showing how different technological tools can help us to understand more about LOF composition, effectiveness, as well as standardizing several aspects during their production process. Owing to their physical, chemical, and microbiological properties, the LOF represent a great tool to address issues of food safety and environmental conservation; however, their application presents some challenges:

1) Standardization of their formulation due to the diversity of raw materials and variability in their composition;

2) Determination of their metabolic composition by the possible presence of unidentified metabolites;

3) Implementation of biotechnological techniques to monitor microbiological quality during anaerobic fermentation and the final product;

4) Accurate quantification of production and application costs compared with agrochemicals;

5) Transfer of technical-scientific information between small and medium producers and the scientific community;

6) Support the development of technological initiatives between research centers and producers through government funds that will allow producers to access technological tools to improve their product quality.

The starting point is to generate knowledge through studies on the characterization, dose, and biostimulant effect per crop; analysis via technological tools such as molecular biology, microscopy, bioprocesses, metabolomics, and metagenomics would facilitate the transition to a more standardized and scientifically based practice.

To understand the effects of LOF, it is necessary to apply scientific information in the field, where changing conditions affect their effectiveness. This requires both the generation of knowledge through studies and the transfer of knowledge to the field, as well as the compilation of results in realistic conditions for analysis in the laboratory. Part of our work aims to bring science to those who are not in contact with it, showing that the artisanal production of LOF has excellent potential to be projected worldwide.

Acknowledgments

The authors are sincerely grateful to the Centro Nacional de Alta Tecnología, Centro Nacional de Innovaciones Biotecnológicas, and the Consejo Nacional de Rectores (CENIBiot-CeNAT-CONARE) for providing funding for the elaboration of this review, as well as the editors and reviewers for their valuable feedback.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

All authors participated in the conceptualization and design of the first draft of the manuscript. AVR, NES, SMB, JUR, JAM, and DRP assisted in the writing of the first version of the manuscript. SMB contributed to the supervision, revision, correction, and final synthesis. EAV participated in the supervision and revision of the manuscript throughout its edition. All authors have read and approved the final version of the manuscript.

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