A review of the state of knowledge and use of liquid ferments and biol in the Andes

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Abbreviations

AMF	Arbuscular mycorhizal fungi
BOD	Biological oxygen demand
C	Carbon
C:N	The ratio of carbon to nitrogen
Ca	Calcium
CH_4	Methane gas
CO_2	Carbon dioxide
Cu	Copper
EC	Electrical conductivity
EM	Effective microorganisms
Fe	Iron
H_2S	Hydrogen sulfide gas
K	Potassium
LAB	Lactic acid bacteria
Mg	Magnesium
Mn	Manganese
N_2	Nitrogen gas (dinitrogen)
N_2O	Nitrous oxide gas
NH_3	Ammonia
NH_4^+	Ammonium
NO_3^-	Nitrate
P	Phosphorus
PGPR	Plant growth promoting rhizobacteria
PSB	Phosphate solubilizing bacteria
S	Sulfur
VFA	Volatile fatty acids
SO_4^{2-}	Sulfate
Zn	Zinc

1. Abstract

Here we review the current state of knowledge and practical use of liquid ferments in the Andes, through an extensive literature review and interviews with practitioners. Literature was collected through searches in white and grey literature, including peer-reviewed research, academic theses and promotional material from non-governmental organizations and government ministries. Interviews were conducted in Bolivia, Ecuador and Peru, in multiple regions and with a range of practitioners, promotors and producers. The basic structure for this review includes: 1) a background of the development of liquid fermented inputs to crops in the Andes 2) critical concepts related to anaerobic digestion and fermentation as it relates to fates of plant nutrients and other end products 3) reporting on the research and promotional literature regarding formulation, chemical properties and application of liquid ferments to crops 4) contextualizing regional literature with knowledge from interviews with producers and practitioners in the Andes, and broader research into bioinput. This final section also includes key recommendations for advancing research into liquid ferments in the Andes. Our review indicates that use of liquid ferments on crops developed in tandem with promotion of household anaerobic digesters for biogas in the Andes. The liquid slurry exiting these digesters is termed 'biol'. Literature and concepts from anaerobic digestion are helpful in understanding the properties and agronomic importance of biol and other ferments. In conjunction with biol from anaerobic digesters, a wide variety of locally-produced ferments are prepared exclusively for application to crops and promoted in the Andes as a low-cost, foliar fertilizers. A large diversity of organic and inorganic inputs are used to make these ferments, though nearly all include manure, which is diluted and decomposed under anaerobic conditions from several weeks to months. The primary agronomic value of these liquid ferments lies in the plant nutrients released from inputs during fermentation. We found large variations in the concentration of plant nutrients in ferments reported in the literature, which were not always closely correlated with the quantity of added manure. Chemical properties such as pH, which varies based on types of inputs and microbial activity, have an overriding effect on the chemical form and fate of nutrients such as nitrogen and phosphorus held in ferments. With decomposition, plant nutrients may be dissolved in liquid, or be associated with remaining solids. We found manure-based ferments may contain agronomically important microbes and organic compounds such as plant hormones which may benefit crops, though the relative importance of these other components is poorly reported in the literature. In the Andes, we found liquid ferments can benefit crop growth, particularly with regular application, though they are not a replacement for other sources of plant nutrients such as compost. We did not find evidence that ferments alter soil microbial communities, but they may stimulate important processes such as nitrogen availability and soil respiration. Along with these benefits, manure-based ferments can carry human pathogens, which are important to consider for household health and commerce of crops. Overall, we found the benefits of liquid ferment application to crops may be improved by standardizing nutrient value of diverse inputs entering the fermentation, optimizing fermentation conditions (including temperature and duration), and maximizing application of nutrients held in liquid and solid fractions. Meanwhile we found promotion of their use was abundant, among development professionals and via local knowledge networks, though we found that deeper knowledge of the complexities of their preparation and use is perhaps limited to a small group of practitioners in the Andean region. Both knowledge regarding liquid ferment use and their chemical properties are highly variable. Nevertheless, those interviewed often had strong views on biol use linked to sociopolitical outlooks as much as from experience with biol as a tool for agroecological nutrient management on small farms. Research and development can adapt them as an important tool for agroecological management in the Andes.

2. Introduction

Effective agroecological techniques for improving soil nutrient management are of crucial importance for resource-limited, small holder farmers in the Andes where recycling nutrients is critical for soil health, to avoid soil degradation, and to buffer against climate and weather extremes. Addition of bioinputs, such as liquid fermentations of manure, are commonly promoted in the Andes to improve crop vigor, alleviate crop damage from frost, combat pests and improve soil quality. Despite their prevalence, little systematized information exists detailing the variety of liquid bioinputs, their preparation, properties or effectiveness. This report seeks to fill this knowledge gap and understand the utility of fermented liquid inputs in agroecological practices in the Andean context.

Organic resources such as manure and crop residue contain nutrients, such as nitrogen and phosphorus, accumulated from across the landscape. Microbial processes in liquid ferments further concentrate these nutrients, and may confer other agronomic benefits. Microbial decomposition of organic matter also releases gases, and under certain conditions can produce biogas for heating and cooking. In the Andes liquid fermentations are often prepared exclusively as foliar crop amendments. The properties of liquid ferments applied to crops depend on factors such as the type and amount of starting inputs, fermentation conditions, and the activity of specific microbes. Careful consideration of these factors, which may vary across the Andes, is needed to better understand the agroecological value of the liquid ferments.

The goal of this work is to collect and analyze information and practical knowledge regarding liquid bio-inputs in the Andes. The assumption underlying this effort is that this knowledge is dispersed among formal literature (peer-reviewed journals), informal sources, (promotional material from NGOs), as well as among farmers and other practitioners in the Andes. In collecting this knowledge, we differentiate among bioinputs, their preparation and application. We conduct a meta-analysis among studies to better understand the properties of liquid ferments in the Andes, and assess the properties, knowledge and use of liquid ferments. Finally, we use regional and global literature to contextualize and identify needs for improved practices and basic research in the Andes.

This report uses two approaches to understand the context and current state of knowledge of liquid bioinputs to small holder agriculture in the High Andes; a thorough literature review of peer-reviewed and grey literature, and a series of qualitative interviews across the Andean region. Findings thus drawn on both academic research, learning by NGOs and other development actors, and the practical knowledge networks of farmers. The first part of the review (section 4) includes a global synopsis of the preparation, properties, and use of liquid ferments used in smallholder agriculture, as well as a focus of the historical and regional context for the current state of knowledge in the Andes.

Next, section 5 defines key terms found in the literature and in interviews. Many of the terms and concepts used in promotional material and field manuals are distinct from those found in more technical research literature. A brief typology clarifies and defines terms which are found in promotional literature or are frequently used in an inconsistent manner among different literature sources.

Understanding properties of liquid ferments requires some technical knowledge. Section 6 of the report details processes occurring in liquid ferments, the role of microbes, and key transformations occurring during the fermentation process. Though not essential to the current knowledge and application of liquid ferments in the Andes, this background is helpful as a reference, and a scientific framework for interpreting findings from the review and in considering future efforts.

Section 7 evaluates knowledge from literature primarily from the Andes as well as from interviews from the region, including how promotional materials, such as field manuals, define 'biol', the role of different inputs and the preparation and use of liquid ferments. Section 8 uses a meta-analysis approach to evaluate chemical properties in liquid ferments across studies in the Andes, and discusses microbiological properties, and how practitioners in the field carry out fermentations. The agronomic impact of liquid ferments is reported in section 9, including effect on plants and soil, how practitioners use biol in the Andes, and includes context from broader regional and global studies into the effects on liquid ferments on crops.

These latter sections include 'Research Insights' - highlighted in yellow boxes - which contextualize findings from the Andes with insights from research findings outside the region. 'Critical summary' - highlighted in green boxes are used to concisely summarize important findings form the literature. Section 10 summarizes results from extensive interviews about use of liquid ferments across multiple regions in Ecuador, Bolivia and Peru. Finally, section 11 discusses knowledge gaps, such as impacts on human health and socioeconomic factors, and compiles overarching conclusions and recommendations from the review.

3. Methods

We used an interdisciplinary methodological approach and sought to establish a dialogue between academic scientific knowledge and empirical knowledge, which comes from traditional knowledge and practice. Thus, the research included two components: a literature review and a socioecological component. The first results of the review contributed to the design of the data collection instruments for the socioecological component. The findings of the socioecological component then fed back into the analysis of the review (Figure 1).

Figure 1. Representation of the dialogue between scientific and empirical knowledge through mutual and iterative feedback during the research process.

The methodological design was both quantitative and qualitative and was developed in stages (Figure 2). First, a search and review of bibliographic sources was carried out to identify information gaps and formulate new questions regarding the use of liquid ferments in the Andes. These preliminary findings contributed to the initial mapping of actors and experiences in the Andean region of Bolivia, Ecuador and Peru, and to the design of the interviews. In parallel, data collection continued for each component and iterative feedback between the two. The methodology used for each component is explained in greater detail below.

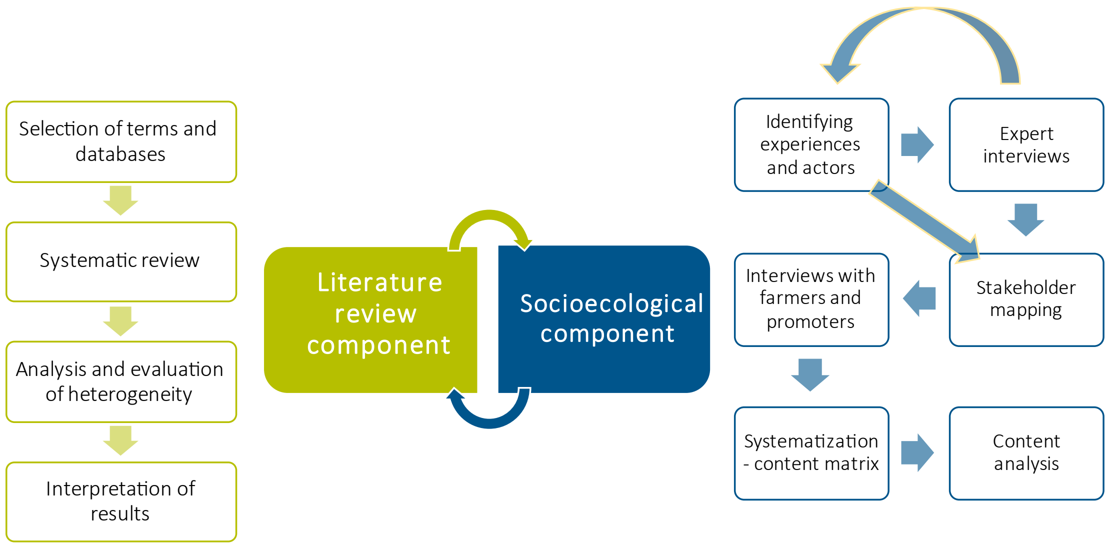


Figure 2. Stages of the methodology of the literature review and socioecological components. The first steps of each process are at the top of the diagram.

A. Literature review

Initial literature searches were carried out on research databases and the world wide web, using specific search terms and followed references cited within the resources obtained from the search. The review includes formal literature such as peer-reviewed research, as well as 'gray' sources such as manuals and reports from NGOs and governments, aimed at practitioners.

Literature relevant to liquid bioinputs was subdivided into distinct categories, including: global background and history, Andean context, biochemistry, microbiology, socioecological components, agronomic usage. Within this broader review we evaluated 123 documents from the Andes or neighboring regions that pertained directly to some aspect of preparation, properties, and use of manure-based fermentations. These sources included promotional manuals, research articles and academic theses (Figure 3).

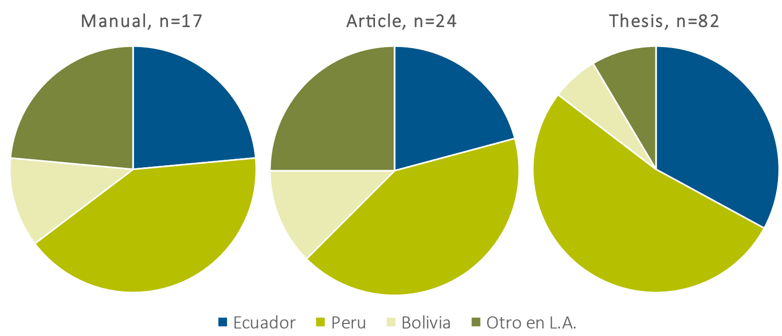


Figure 3. Origin of the regional literature evaluated in this review, including manuals, research articles and academic theses (the number of each appears next to the literature type) from Bolivia, Ecuador, Peru, and other parts of Latin America (L.A.).

Almost half of these documents originated from Peru, followed by Ecuador and Bolivia. Where relevant we also included literature from other parts of Latin America, including Chile, Colombia, Brazil, Costa Rica, Venezuela and Mexico. The scope of information and data varies by source. Some literature contains detailed explanations of the ingredients and preparation of fermentations, but little on application and use. Other sources do not describe the source or properties of the biol, but describe effect on crops.

Qualitative information, such as ingredients, preparation, and application from regional literature sources were compiled and summarized in tables. Where possible, we extracted quantitative data directly from published resources, including quantities of materials added to ferment preparations and properties (i.e. pH, nutrient concentrations) and used a meta-analysis approach to draw quantitative findings across the literature. Where quantitative literature was not directly comparable among sources - effects on different crops, pests or agronomic parameters - we summarize findings in tables. We limit or exclude use of quantitative data from sources lacking detail or clarity in experimental design and methodology.

B. Interviews

The socioecological component was approached using a qualitative approach consisting of semi-structured interviews with key stakeholders in the Andean region of Bolivia, Ecuador and Peru. Eight representative Andean provinces were selected according to the following criteria (a) altitude variability; (b) type of production and agricultural practices; (c) presence of producer associations and/or development projects that promote or promoted agroecological practices. Thus, the following were chosen: Cochabamba, La Paz and Oruro, in Bolivia; Pichincha and Azuay, in Ecuador; and Ancash, Huanuco and Lima, in Peru (Figure 4).

LINK

Figure 4. Location and altitude of the field of action of the farmers and promoters interviewed in Bolivia, Ecuador and Peru. The interviewees are located between 1800 and 4500 meters above sea level.

Four interview formats were designed for different actors: technical experts, promoters or field technicians, farmers who use biol and farmers who do not use biol. The design of the questions considered the initial findings and the information gaps identified in the literature review, seeking a complementarity between both types of knowledge (scientific and practical-empirical). The questionnaires for promoters and producers consisted of approximately 70 open-ended questions covering three categories of qualitative variables: practical knowledge, knowledge transmission and sustainability of the practice.

We applied a non-random sampling, by accessibility. Data collection was carried out in three moments: a) interviews with experts from each selected province, who provided us with information on the context and agrarian dynamics of each territory, as well as recommendations of experiences (development projects, small-scale factories), of field promoters and leaders of associations to be interviewed; b) interviews with field promoters and leaders of producer associations; 3) interviews with farmers, who were identified with the support of promoters and leaders (Figure 5). In addition, seven farmers who abandoned the use of biol in the selected cases were interviewed. Interviews were conducted until theoretical saturation was reached. The conceptual model (Figure 5) presents the selection process of the interviewees.

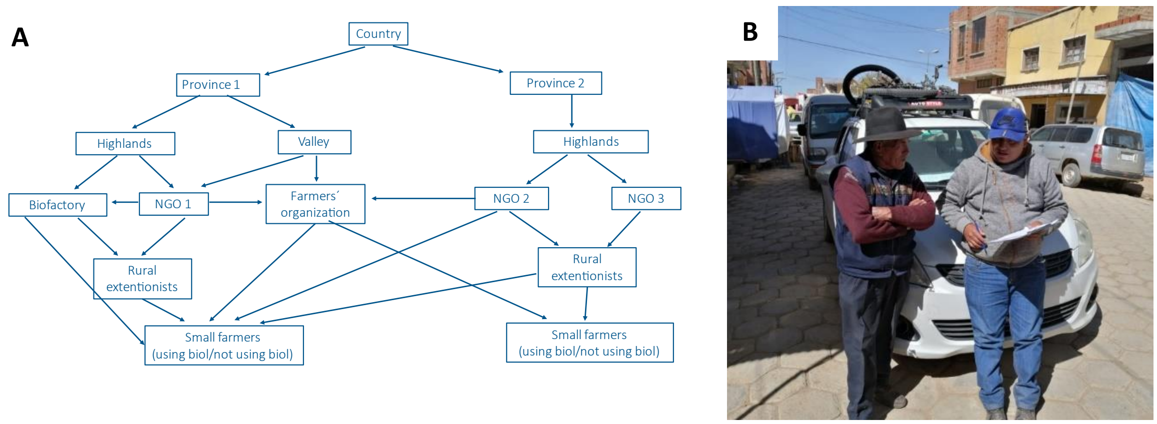


Figure 5. Conceptual model for the selection of interviewees in the eight provinces evaluated ***(A).*** *Focal point in Bolivia conducting an interview with a producer* ***(B).***

Between March and August 2020, 111 interviews were conducted (25% women), of which 25 were in Bolivia, 39 in Ecuador and 47 in Peru. Figure 6 shows the territorial and gender distribution of the interviewees. Initially, field visits to farms and premises of farmers' associations were planned, but this activity was suspended due to restrictions brought about by the COVID-19 pandemic. Most of the interviews were conducted remotely (by telephone, WhatsApp and Zoom). For this data collection, we had local support staff in each country. Each of them had a good knowledge of the use of biols and the actors involved in the provinces evaluated. The interviews were transcribed and then the information was entered into an excel matrix of variables. Subsequently, a selection of the most relevant categories was made, the responses were reduced, and the final analysis matrix was constructed, concluding with the content analysis for the interpretation of the results.

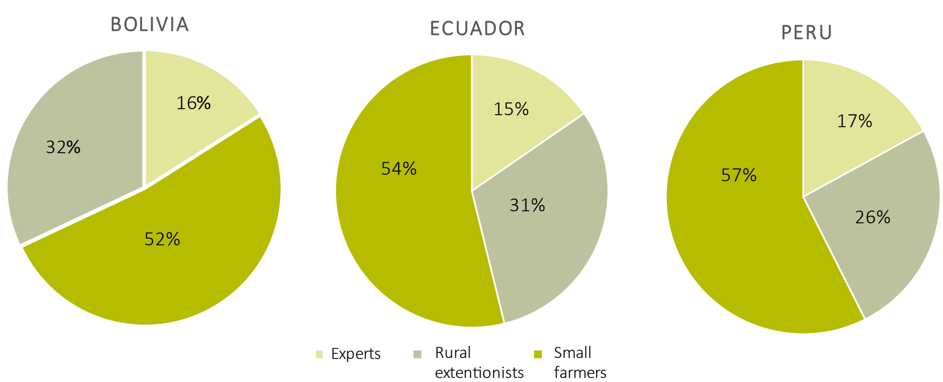
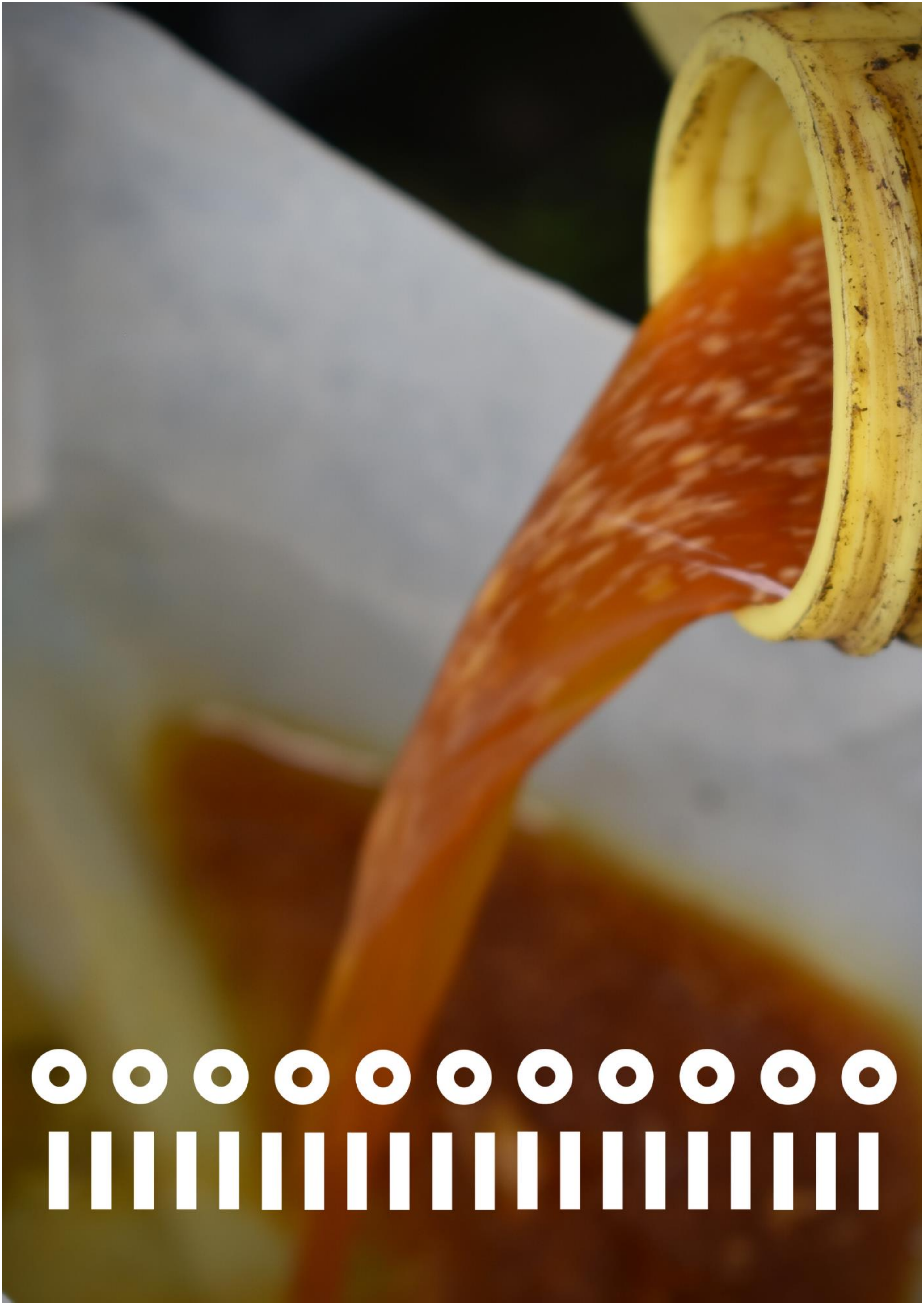


Figure 6. Percentage distribution of the type of actors interviewed in Bolivia, Ecuador and Peru.



4. Global and regional context for the development of liquid bioinputs

The use of liquid ferments in small-scale agriculture occurs worldwide, with different regional and historical influences in Latin America and the Andes. Perhaps the most influential factor is the promotion of biodigesters to produce biogas which, in turn, generates energy in isolated areas of the Andean region. A second factor is the implementation of development projects, carried out by NGOs, that promote fermented liquid fertilizers exclusively as agronomic inputs to support small producers. Although anaerobic digestion and on-farm fermented liquid fertilizers are different, both use microorganisms to break down organic matter and release plant nutrients in fermentation. Another important influence is the increase in research that recognizes the importance of microorganisms in soil and plant health. The prevalence of microbes in liquid ferments has been used to suggest, often explicitly, a link to microorganisms that have been shown to interact directly with plants to promote their growth.

A. Anaerobic digesters in rural communities and use of effluent in crops

The emergence of liquid ferments in Andean agriculture has its origin in the promotion of domestic biodigesters used for cooking and providing heat. This process began in the late 1970s with the onset of the global energy crisis, which resulted in a drive to seek alternatives to fossil fuels. Anaerobic digesters use biological waste, such as animal manure, food scraps or municipal waste, in a controlled decomposition process in the absence of oxygen to support microorganisms that generate biogas, such as methane (CH_4). A by-product of this process is an effluent rich in plant nutrients.

The basic infrastructure of an anaerobic digester is a lined and covered pit, or sealed vessel, used to house the microbial processes and capture biogas. Most biodigesters are designed to operate continuously, with an inlet for adding inputs and an outlet for the liquid waste. The composition and rate of input addition are optimized for biogas production. The first simple biodigesters were manufactured in Asia, so the designs are often characterized as "Indian," "Chinese," or "Taiwanese...". (Pérez et al. 2014, Garfí et al. 2016).

In the mid-1980s, Chinese "fixed-dome" digesters made of bricks and concrete were widely promoted in Latin America. In the Andes, national programs funded by European non-governmental organizations (NGOs) supported this effort. Northern European countries have a history of experience in biogas production, and an explicit effort was made to transfer this knowledge to the Andean region. (Gruber and Herz 1996). At that time, 65 small-scale biodigesters were built in Bolivia and more than 100 in Peru. All but one of these biodigesters ceased operation due to lack of ongoing training and assistance. However, these projects also documented how small farmers were using the effluent from the biodigesters directly on the fields with visible improvements in crops. (Garfí et al. 2016).

The diffusion of Taiwanese "tubular" biodigesters, made of plastic, grew through grassroots agroecological efforts with early work in Colombia (Botero and Preston 1987). Tubular digesters (Figure 7) are more common in the Andes as they are sometimes housed inside a specially built structure to stabilize temperatures at higher altitudes. These designs have a low initial cost and are easy to install. The liquid slurry by-product, or "biol," was promoted as a resource to increase crop production. (Martí-Herrero et al. 2014). In the Andes, national biogas programs and associated NGOs extolled the benefits of digester biol as a soil amendment. (Acosta et al. 2013, Warnars and Oppenoorth 2014, Martí-Herrero et al. 2014). In fact, the cost of a domestic biodigester was justified by the possibility of reducing the purchase of fertilizer for crops. (Arrieta Palacios 2016).

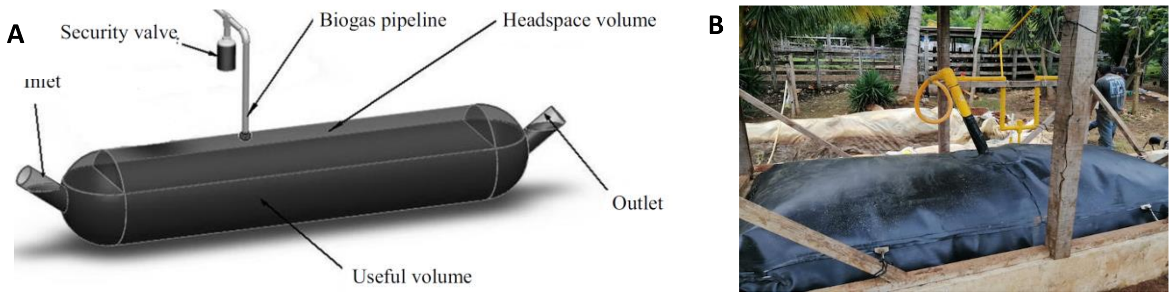


*Figure 7: Schematic of a basic design of a tubular-type digester, taken from Garfi et al. (2016) (**A**). An image of a working tubular digester from https://www.permaculturenews.org/2020/10/23/biogas-and-carbon-farming-part-3/ml (**B**).*

Despite initial challenges, support for the development of household digesters continues. The Biodigester Network for Latin America and the Caribbean (RedBioLAC) consists of 55 partners, including NGOs, research centers and companies, representing 18 countries. (Garfí et al. 2016). RedBioLAC holds regular conferences to advance biogas development and the use of biol from digester effluents on crops. As of the mid-2010's, national programs continue to advance plans to build tens of thousands of household digesters in Bolivia, Peru and other countries in Latin American (Garwood 2010, Garfí et al. 2016).

B. The agroecological movement promotes liquid ferments as fertilizers

In Latin America, the development and promotion of liquid ferments produced exclusively as crop amendments gained momentum in the 1990s. Unlike most biogas digesters, which operate continuously, these ferments are produced in batches, in simple containers. Practices using fermented manure-based amendments on Andean crops have ancient, global and regional origins.

The use of liquid ferments on crops has deep roots in South Asia, with dung, milk, and fish-based preparations described in Vedic texts dating back to before 1000 BC. (Gore and Sreenivasa 2011, Sarkar et al. 2014). Some ancestral techniques such as Bokashi - a Korean practice of fermenting food waste for soil application - were widely promoted as an agroecological practice in Latin America. (Garro Alfaro 2016). These techniques are similar to the fermentations used to preserve food and produce silage for livestock: air is excluded to induce microbial fermentation of sugars that partially decompose into acids that then act, in part, as preservatives. In this sense, it is worth mentioning that fermented beverages are common in the Andes. Chicha is obtained from the fermentation of corn kernels, using sugars such as chancaca to accelerate fermentation. Although not applied to crops, chicha was traditionally used in ceremonies to promote good harvests. (van den Berg 1989).

In the Andes, pre-Hispanic cultures used guano, primarily from deposits in sea-bird rookeries, to improve crop productivity. Guano is a natural fertilizer with high phosphorus and nitrogen content, which was collected and added to the crop. This practice supported large pre-Inca populations as early as 1000 AD. (Santana-Sagredo et al. 2021) and the trade in guano fueled the intensification of agriculture in the region and beyond, both before and after the colonial period (Cushman 2017). Although not a direct analogue of contemporary liquid ferments, guano is a manure amendment for soil fertility that is still promoted through the agroecological movement in the Andes.

A direct link to liquid ferments used in Latin America comes from Brazil since the early 1990s. Farmers and rural extension specialists combined recalcitrant organic residues, such as sugarcane bagasse, with bovine manure and added energy-rich carbon sources (e.g., molasses, milk) to stimulate fermentation. Mineral salts, such as calcium chloride and copper sulfate, are added after early fermentation. One experienced user, Delvino Magro, applied the appellation "super," adapted from industrial fertilizer formulations, resulting in the popular liquid foliar fertilizer known as "Super Magro" (Oliveira et al. 2017).

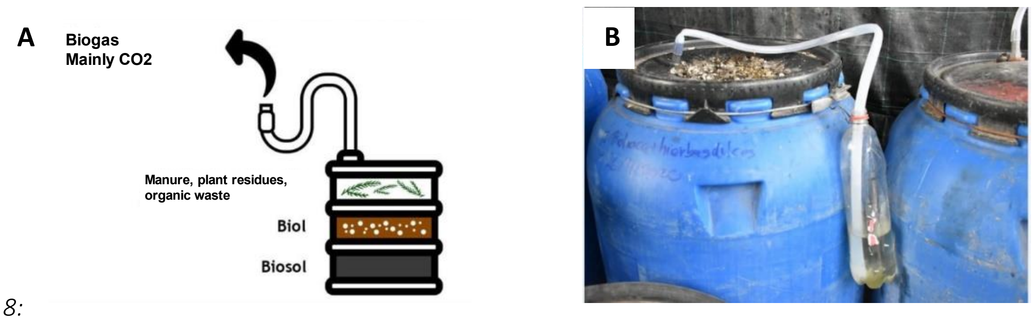


Figure 8:
*Schematic of a basic design of an on-farm liquid fermentation unit **(A)**. An image of a fermentation vessel in operation in the Andes **(B).***

Similar preparations appear in manuals and popular literature throughout Central and South America, which emphasize the adaptation of recipes to locally available resources, resulting in dozens of formulations designated as "biopreparation", "biofertilizer", "bioferments" (Restrepo 2001, Garro Alfaro 2016). Most of these fermentations have manure as the main component, but vary widely in terms of additional ingredients, such as molasses, rock dust, urine, wood ash, guano, plant biomass, and various minerals and salts, prepared in simple containers (Figure 8). Often techniques are mixed, combining manure fermentation with locally sourced microbial inoculants, or with mineral preparations similar to those prescribed for more conventional foliar fertilizers (Melendez and Molina 2002). Over time, many of these manure-based fermentations came to be referred to as 'biol' in promotional literature, just as slurry from anaerobic digesters (Gomero Osorio 2005). Table 1 lists some basic distinctions between the two.

Table 1. Comparison of anaerobic digester products and liquid ferments prepared exclusively as fertilizers, including materials, processes involved and products.

	Anaerobic digest for biogas	Farmmade liquid ferment
Material	• Simple waste stream . E.g. manure with whey • Optimizaçe ingredients for biogas production	• Often complex: manure, plants, inorganics, sugars , etc. • Optimize for rapid fermentation , agroecological properties
Process	• Strictly anaerobic • Continuous operation	• Mostly anaerobic conditions • Simple "batch" fermenter vessel
Product	• Biogas (i.e. CH4) • Liquid slurry with neutral pH • High in plant nutrients and recalcitrant organic matter	• Slurry to neutral to acid pH • High in plant nutrientes and recalcitrant organic matter

C. Recognition of beneficial microbes for soil and plant health

Another factor leading to the promotion of liquid ferments as crop amendments is research demonstrating the importance of microorganisms in soil fertility and plant growth. Specific microorganisms stimulate plant growth through nitrogen fixation, plant hormone production, disease suppression, and other mechanisms, prompting interest in the development of microorganisms as biofertilizers (Vessey 2003). This includes research in the Andes, such as the isolation of phosphate-solubilizing bacteria (Pérez et al. 2007, Pandey and Yarzábal 2019) and enrichments of fungal metabolites that stimulate quinoa growth (Ortuño et al. 2013).

Recognition of the importance of microorganisms in plant growth inspired the development and commercialization of "efficient microorganisms" (EMs)-mixes of microorganisms that are purported to promote growth when applied to plants (Higa 1989, Singh et al. 2011). EMs range from commercial preparations, whose origins vary widely, to home-made enrichments that use soil as the initial inoculant. Many fermented liquid fertilizer preparations include inoculums (e.g., commercial or soil-derived EMs) and are promoted as having a *de facto* enrichments of specialized plant growth-promoting microorganisms.

Liquid ferments have high concentrations of plant nutrients that stimulate plant growth. However, the explicit linking of plant growth-promoting microorganisms to liquid ferments manufactured on farms has furthered their mystique as having additional special properties. These claims permeate literature promoting liquid ferments. However, research efforts developing biofertilizer inoculants generally focus on ecological interactions between specific microbial strains and crop cultivars, including those adapted to agroecosystems in Latin America (Peña Borrego et al. 2015). We found no study demonstrating positive impact on plant growth due specifically to microbes found in manure-based ferments that is clearly distinct from the effect of added plant nutrients in ferments.

5. Typology of liquid preparations applied to soil and plants.

The development of anaerobic digesters for biogas, with the use of the effluent "biol" on crops, had a strong influence on the promotion of liquid fertilizers in the Andes. Parallel to this process was the expansion of liquid ferments used exclusively on crops, which gained momentum through experienced users and promoters of agroecology in Central and South America (Restrepo 1998). The terms and concepts related to these practices are distinct, but both academic and promotional literature in the Andes reflect the mixing and overlapping of terminology.

In addition, several other liquid inputs - both commercial and home-produced - are promoted as stimulating plant growth. For example, foliar fertilizer blends with micronutrients use chelating agents[1] to enhance nutrient uptake into leaves (Melendez and Molina 2002) which can help plants under abiotic stress (i.e., excessive heat or freezing). Liquid broths with sulfur minerals can combat plant pests (biotic stress). Liquid suspensions[2] of wood ash can have both nutritive and anti-pest properties (Suquilanda 2012). This review does not focus on these numerous formulations which rely on inherent chemical properties for effectiveness, but on liquid 'ferments' which undergo microbiological processes in their preparation. Liquid ferments may be used together with other inputs such as those noted above, and many liquid ferments may possess some of the biostimulative properties of non-fermented formulations. That said, the fact that ferments undergo microbiological processing does not contradict that their effectiveness could be due partly or wholly to chemical composition, such as plant nutrients.

In this section we offer a typology of terms found in the literature, both to familiarize the reader with the most frequent uses and to clarify concepts. In addition to delineating the terms that appear in the present review, this will aid in the discussion of the microbiological and chemical processes that occur in ferments. Greater clarity is needed if the current state of knowledge on liquid ferments is to be translated into practical research and application in smallholder agroecosystems.

Figure 9 provides a visual typology of terms commonly found in the literature and describes the relationship between them. A plant biostimulant simply provides some positive impact on plant growth and is the broadest category that encompasses all other inputs. A bioinput is a biostimulant, but derived from some organic source (i.e., not a mineral fertilizer), such as a plant extract or wood ash. The term "bioferment" appears widely in promotional literature and indicates that a fermentation process has been used to produce a bioinput (such as a compost tea). A biofertilizer is a microbial enrichment, either a pure isolate of a single microorganism or a consortium of microorganisms used as an inoculant. A biol is the product of a mainly manure-based fermentation produced under anaerobic conditions. A mineral broth is a dilution or suspension of minerals (not necessarily organic) that is a biostimulant but can also be incorporated into a bioferment.

[1] Chelating agents: compound that binds metal ions (such as minerals) and prevents them from interacting with other ions. In foliar fertilizers, they help to retain the plant-available form of micronutrients for better absorption into plant tissues. Chelating compounds are produced industrially and are widely present in nature.
[2] A suspension is a fluid containing solid particles, visible or microscopic, which are not dissolved in solution and can therefore settle out over time.

Relevant terms from the literature review are presented below. In some cases, terms are used inconsistently across sources. Therefore, we provide more context, for example, as to how they are used in the research literature compared to the advocacy literature in the Andean region.

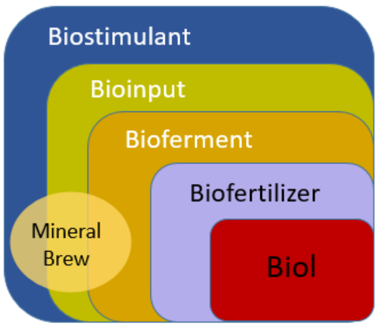


Figure 9. Representation of the relationship between terms commonly found in the literature on liquid preparations applied in agroecosystems. A biostimulant is the broadest designation - all other preparations are biostimulants. Terms bounded by smaller shapes have more specific distinctions - for example, a biofertilizer has a narrower definition than a bioinput. A biol has properties of all preparations, as a product of fermentation of organic inputs, with an abundant microbial population, and applied to stimulate plant growth. Mineral broths are liquid preparations, and although they are not fermented, the literature reflects that they can be mixed with other ferments.

Anaerobic digestion - The process of converting waste - household waste, manure, crop biomass or other organic sources - by harnessing a series of microbial processes that convert complex organic matter into biogas, such as methane. The remaining liquid sludge (biol) and solids (biosol) are often used as fertilizer, while the methane can be used as fuel which also negates its enhancement of global warming.

Biogas - Gases produced by microorganisms from the decomposition of organic matter under anaerobic conditions. The dominant biogases are methane (CH_4) and carbon dioxide (CO_2), but also include ammonia (NH_3), nitrogen gas (N_2), nitrous oxide (N_2O), hydrogen (H_2) and hydrogen sulfide (H_2S).

Bioinput - An amendment applied to a plant or soil, derived from plant, animal, or microbial material, either fresh or processed prior to application, including plant or plant extract, animal excrement, or compost. The proposed use may include enhancement of beneficial soil processes, fertilization, stimulation of plant hormones, control of antagonists such as plant pests, or some combination of these.

Biostimulant - In a broad sense, products derived from organic or inorganic substances or microorganisms used to enhance plant growth or alleviate biotic/abiotic stress. They include minerals, hormones, vitamins and other organic compounds. In many cases, the mode of action on plant response may not be known or understood. See Bulgari et al. (2019)and for historical and global context, see Yakhin at al. (2017).

Biofertilizer - Preparations of microorganisms, either individual strains or consortia, that are applied to seeds, plants or soil and are intended to stimulate crop growth, (Vessey 2003, Peña Borrego et al. 2015). This term is also commonly used in a broad sense in the literature to include many bioinputs, such as compost.

Bioferment - Appears mainly in promotional literature and refers to a wide range of fermentations of either plants, fertilizers or other ingredients, intended for application to crops and soil.

Biol - Liquid part of a fermented preparation whose main component is animal manure. It is used as a foliar amendment. Originally the term described the liquid effluent from the anaerobic digestion of animal manure to produce biogas. It is also a manure-based preparation, usually with other inputs, anaerobically fermented for 1 to 3 months, made exclusively for use on crops to improve plant growth.

Biosol - Solid sediment remaining after fermentation to produce a biol. Biosol is high in plant nutrients and can be applied as a soil amendment.

Chelates - Suspension or dilution of minerals or metals (e.g. iron, zinc, copper) mixed with chelating agents, applied directly to plants or combined with fermentations.

Digestate - Slurry effluent from an anaerobic digester, often applied to land as a soil fertility amendment.

Efficient microorganisms - Commercial mixtures of microbes sold in liquid concentrates and marketed as containing microorganisms such as lactic acid bacteria (LAB), yeasts and photosynthetic bacteria, intended to benefit plants through activities such as nitrogen fixation, plant hormone production and disease suppression.

Fermentation - In general terms, fermentation describes the breakdown of organic matter, carried out by enzymes usually derived from microorganisms, usually in the absence of oxygen. Fermentation also refers to a specific metabolic process that releases energy to cells from the partial breakdown of sugars into organic acids, in the absence of oxygen and with the release of CO_2 gas. (For the purposes of this review, we refer to fermentation in the broad sense, unless otherwise specified).

'Mountain' or 'wild' Microorganisms - This is a microbial enrichment derived from the collection and fermentation of soils, often from less disturbed soils (e.g. from forests), with the idea that these contain microorganisms with uniquely beneficial properties for crops and/or for their activity in fermentations.

Mineral stock - A liquid mineral dilution or suspension, containing ingredients such as rock dust, ash, sulfur and other industrial salts. Once mixed, it is added directly to plants as a nutrient, to combat pests or pathogens, or to stimulate growth.

6. Microbial and biochemical processes in liquid ferments

The basic process occurring in liquid ferments is the decomposition of complex organic matter[3] by microorganisms. This decomposition results in the production of biogas (such as CO_2 and CH_4) which can leave the ferment. As carbon is removed, this releases and concentrates plant nutrients, such as nitrogen (N) and phosphorus (P). The steps of anaerobic digestion are well studied and are a useful guide to understanding liquid ferments, including those originating from small batches made on farms.

A. Anaerobic digestion and liquid fermentation: basic processes and key steps

Organic matter used in anaerobic digestion, such as animal manure and plants, is chemically complex. These materials contain mixtures of starches, fibers, fats and proteins, as well as small amounts of metals and salts, such as potassium (K), magnesium (Mg), calcium (Ca) and iron (Fe). In liquid ferments, microorganisms produce enzymes that break down this mixture, similar to the way food is broken down in the stomach. Once the enzymes break down the complex molecules, the smaller units can be consumed by the microbes for energy. As the organic matter breaks down, it also releases nutrients such as N, P and sulfur (S) into the liquid ferment. More resistant compounds like cellulose or lignin may remain undigested.

Humans use the chemical energy stored in the carbon bonds in our food. This energy is usually captured in the presence of oxygen, or "aerobically". The oxygen we breathe helps transfer energy from food to our bodies through a process called aerobic respiration. A by-product of this process is the CO_2 that we exhale. Microorganisms carry out the same process when they break down organic matter to harvest the energy it contains. When there is no oxygen - conditions are anaerobic - microbes can capture only part of the energy from these bonds through a process called fermentation. The by-products of this process are partially-consumed, smaller organic molecules and CO_2 .

Microbes can repeat this fermentation process, partially consuming these smaller organic units for energy and releasing more CO_2. Once these molecules are small enough, a different set of specialized microbes consume them for energy and methane gas, CH_4, is released as a byproduct. As more energy is consumed from the starting material, more of the original carbon is lost in the form of CO_2 and CH_4 gas and microbial activity slows down. What remains in the ferment is some undigested organic matter, both solid and dissolved in the ferment itself, as well as minerals and nutrients (i.e., Ca, Fe, K, P, N) that enter the liquid ferment and become more concentrated during the fermentation process.

Anaerobic digestion is a multi-step process carried out by microorganisms in the absence of oxygen. Many of the steps of anaerobic digestion for biogas production can also occur in other types of fermentations, such as those that occur during the on-farm liquid fertilizer production process. In general terms, the chemical energy stored in the organic matter is released by the microbes that use it to grow and reproduce. The key steps in this transformation are hydrolysis, acidogenesis and methanogenesis (Figure 10).

[3] Organic - One meaning refers to material remains of biological origin, which may include carbon, nitrogen, phosphorus, sulfur and other elements. This first meaning is the basis of "organic" agriculture. A second meaning refers to any chemical compound - regardless of its origin - containing carbon and hydrogen bonds, such as sugars and methane gas (CH_4), but not to "inorganic" compounds such as ammonia (NH_4^+) or phosphate (PO_4^{3-}).

1) *Hydrolysis*: Organic matter, such as polysaccharides (cellulose, starch) and proteins, are broken down by enzymes. Polysaccharides are broken down into sugars (e.g. glucose, lactose) and proteins into amino acids. This process requires energy input from the microorganisms that produce the enzymes.

2) *Acidogenesis*: Sugars released by enzymes during hydrolysis are consumed by microorganisms for energy and growth. Acidogenesis produces CO_2 and small organic acids (e.g. lactate, propionate, butyrate) called volatile fatty acids (VFA), which are subsequently consumed to produce even smaller molecules of acetic acid, more CO_2 , as well as hydrogen gas (H_2) in the process.

3) *Methanogenesis*: Specialized microbial processes that consume small carbon molecules, such as acetic acid or CO_2 , for energy and growth, producing CH_4 as a by-product.

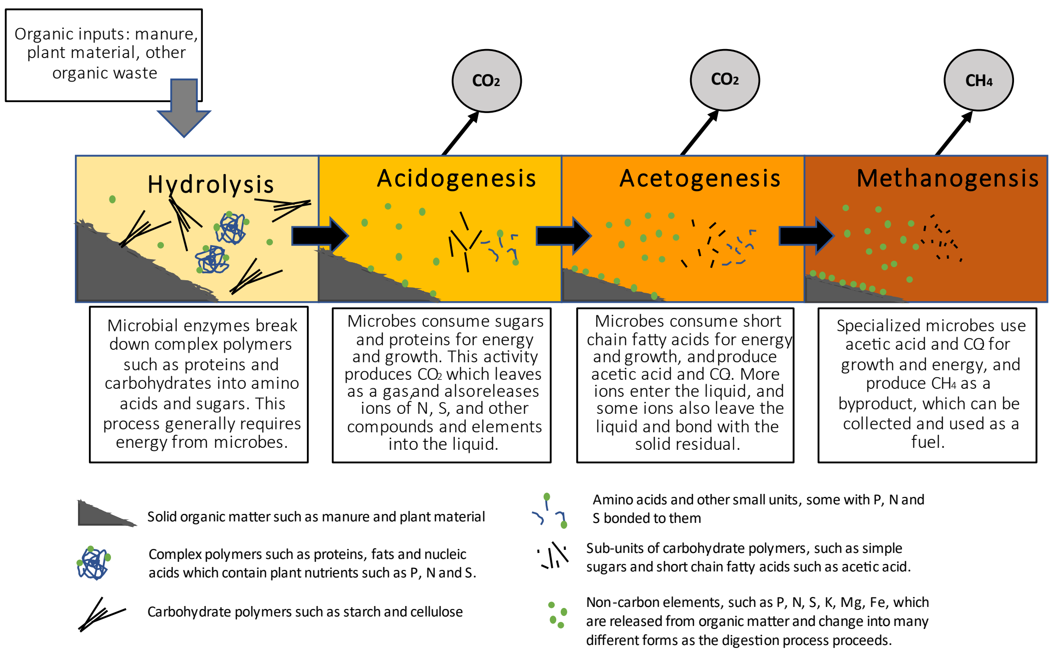


Figure 10. Steps of anaerobic fermentation.

Conditions in an anaerobic digester must favor each of these steps to produce CH_4 . Hydrolysis can occur with or without the presence of oxygen. But fermentation and acidogenesis require anaerobic conditions. Methanogenesis needs anaerobic conditions as well as near neutral pH[4]. Inputs and on-farm biofermenter manufacturing processes may not be optimized for all of these steps. For example, acidogenesis produces VFA, which acidify the ferment (lowering the pH as low as 3.5). If acidic products accumulate faster than they can be consumed, methanogenesis will slow down or stop. Indeed, if the end product is too acidic, this would be similar to the processes used to preserve food by pickling or to produce silage for livestock.

[4] pH - A measure, on a scale of 0-14, of the concentration of protons, H^+ , in a solution. Neutral pH is set at 7. As pH falls below 7, solutions become more acidic. As the pH increases above 7, solutions become more basic.

B. Microbes in liquid ferments

In addition to fermentation and methanogenesis, certain microorganisms can carry out other reactions under anaerobic conditions. For example, many microbes can use alternatives to oxygen, such as nitrate and sulfate, to transfer energy from organic matter. This is called anaerobic respiration, which produces gases such as nitrous oxide, nitrogen and hydrogen sulfide (which has the notable odor of rotten eggs). In addition, many photosynthetic bacteria grow without oxygen. They use light for energy instead of organic matter and some can also carry out nitrogen fixation. (Bryant and Frigaard 2006). These seemingly exotic processes illustrate the complexity of anaerobic liquid ferments and the variety of possible end products (i.e., different gases).

In practice, the basic steps of anaerobic digestion produce biogas and concentrate plant nutrients released from organic matter. The microorganisms that thrive in liquid ferments are highly effective at consuming carbon from complex sources, such as manure, through fermentation or anaerobic respiration. In biogas digesters, this means optimizing the microbes to channel carbon from the starting material into methane gas. In the case of on-farm artisanal biol, this means optimizing conditions to obtain end products that benefit agroecosystems.

Liquid manure-based ferments can contain thousands of different microbial species. (Amani et al. 2010, Li 2013, Sun et al. 2015). The composition of microbes varies greatly among different anaerobic digests, especially based on inputs (Zhang et al. 2014). Microbes introduced into digests are generally consumed during fermentation, and microbial diversity tends to decrease (Mei et al. 2016). Furthermore the microbial communities in digests shift in response to even small changes in inputs and conditions (Hagen et al. 2014).

Microbes isolated from biogas digesters include those with traits that benefit plant growth, such as N-fixation and phosphate solubilization (Ndubuisi-Nnaji et al. 2020). However, DNA sequencing reveals that those with traits that promote plant growth may represent less than 1% of the microorganisms in the digesters. (Coelho et al. 2020). Traits such as N fixation and phosphate solubilization are energetically costly for microorganisms, and may not confer a competitive advantage when N and P are abundant, as is the case with fermentations that have manure as a major component.

Digests of manure and plant residue also contain an enormous diversity of organic compounds, including tannins, terpenes, lignins, proteins, and plant hormones such as indolacetic acid, gibberellic acid and abcisic acid (He et al. 2022). These compounds may be contained in manure itself or in plant residues, and may be concentrated during fermentation just as plant nutrients are (Li et al. 2016b). Microbes may also form precursors of plant hormones such as indolacetic acid (Li et al. 2018). Microbes also produce an array of volatile organic compounds which may play a role in plant health and physiology (Bitas et al. 2013), and while some of these chemical interactions may be highly specific (Isah and Isah 2019), their interaction with the plant microbiome, pathogens, pests or with stress response pathways are poorly understood though critical to plant development and health (Ortíz-Castro et al. 2009, Pang et al. 2021). We found no studies where the effect of these diverse compounds were isolated after the addition of manure-based digests to soil and crops.

As with most organic waste stream applied to crops, liquid ferments of manure can pose a health risk for contaminating water and food with human pathogens, such as fecal coliforms, although digestion may also reduce their abundance (Lansing et al. 2008, Groot and Bogdanski 2013, McCord et al. 2019). Standards for use of manure and greywater in agriculture are established by the World Health Organization (WHO 2006).

Biological oxygen demand (BOD) is used to assess the potential for waste to harbor pathogens. BOD is a measure of the amount of organic matter in a liquid based on the amount of oxygen needed to decompose this material. The lower the BOD, the less decomposable material remaining with the potential to sustain pathogen growth. Since manure-based ferments have high BOD, reducing risks begins with management of manure streams prior to fermentation for safe household use, crop production, or marketing of produce.

C. Inputs and operation

Knowing which organisms are present in a liquid ferment is not a clear indicator of microbial processes at work. Instead, optimizing microbial activity in liquid ferments starts with feedstocks. The relative amount of carbon to nitrogen - the ratio of C:N - is a key factor that varies widely, from 10:1 in chicken manure, 15:1 in cow manure, 20:1 in vegetable and fruit to 80:1 in maize stover (Xu et al. 2018). High C:N feedstocks (those with abundant carbon) tend toward slow fermentation. Meanwhile, excess N (low C:N) inhibits methanogensis, and is not desirable for biogas production. In addition, mixing inputs can sometimes influence nutrient release unpredictably. In one example, manure mixed with legume leaves yielded significantly higher plant-available N and P in final liquid ferment than with manure alone, even though the manure contains far more N and P than the leaves (Kataki et al. 2017).

The properties of inputs can also influence the fermentation conditions, particularly the pH over the course of the fermentation. During fermention, production of VFAs can rapidly acidify the ferment. The capacity of a liquid to buffer changes in pH, such as rapid acidification, is called alkalinity. Substances which increase alkalinity include bicarbonate, phosphate and ammonia which may occur in some inputs (manure) and not in others (refined sugars). Alkalinity varies in inputs (including water from different sources) which can be assess prior to, and over the course of, fermentation as organic material breaks down.

Fermentation processes can be monitored in numerous ways. Odor indicates microbial processes - VFAs have a strong odor ranging from sour to rancid. Color change has also been used to assess fermentation. Low pH (more acidity) reflects acidogenesis processes, which can inhibit methane production. Alkalinity varies widely with feedstocks - while low pH reflects progress in fermentation, high alkalinity will result in small observable changes in pH. A low-cost way to assess both acidity (VFAs) and alkalinity is measuring electrical conductivity[5] (EC) (Aceves-Lara et al. 2012, Robles et al. 2016). Many componds dissolved in the ferment, including VFAs, ammonia, metal and salt ions, carry a small charge which contributes to EC. As organic matter breaks down, EC might be expected to increase and can used to monitor the concentration of key compounds (Charnier et al. 2016). The EC can also be used to assess how much to dilute liquid ferments prior to addition to crops (Möller and Müller 2012) as solutions with high EC (e.g. salt water) harm plants by interfering with how plant roots regulate water intake.

Given the high variability in feedstocks (including water source, and manure type) metrics such as pH, alkalinity and EC are expected to vary widely among biol preparations – their utility as indicators is in how they change over time, from the start to the completion of the fermentation. Note that continuously operating anaerobic digesters aim for stable values for metrics such as pH, alkalinity and EC to optimize

[5] Electrical Conductivity - The capacity of a liquid to conduct an electrical charge. It reflects the total charged solids dissolved in a solution, including VFAs, ammonia and elements such as potassium (K^+), sodium (Na^+), calcium (Ca^{2+}).

conditions for biogas production. By contrast, for batch fermentation systems like farm-made biol, one would expect these indicators to change over time reflecting the course of a complete fermentation.

D. Nutrient transformations during and after fermentation

Regardless of the specific microbial processes at work, during anaerobic digestion and liquid fermentations 20-95 % of carbon is lost, as CO_2 and CH_4, and nutrients, such as N, P, K, S, Ca, Mg and Fe are concentrated in the remaining material (Möller 2015). Some of the original organic material, such as the structural material in plant cell walls like lignin and cellulose - is resistant to breakdown and will remain in a solid form. As a result this 'undigested' material - called 'biosol' in the Andes – remains high in carbon, still contains unreleased nutrients, and also influences the properties of the ferment.

The nutrient content in the liquid ferment depends on the nutrients in the starting material (Insam et al. 2015). At the end of the fermentation, the concentration and chemical form of nutrients will depend largely on the pH, alkalinity and redox[6] (Möller and Müller 2012, Mao et al. 2015). Indeed the loss of more carbon as CH_4 (anaerobic digestion), compared to CO_2 (in farm-made fermenters) likely affects the chemical properties of the liquid ferment. The forms of nutrients can continue to change after fermentation and during storage (Bonten et al. 2014).

The case of N illustrates how products of fermentation can vary. Some N can be lost as gas. Most N released during fermentation dissolves into the ferment liquid as ammonia (NH_3), which is highly soluble[7] and is easily availabe to plants compared to N held in starting material such as manure. As the ferment becomes basic (above pH 7), more and more NH_3 can be lost as a gas (notable as the strong ammonia smell of household cleaner or urine). At low pH, ferments can retain more NH_3, but more can also precipitate as a solid such as ammonium carbonate (Möller and Müller 2012).

Other nutrients, such as potassium, are more likely to remain dissolved in the liquid ferment and not change form. Phosphorous released from organic matter during fermentation does not leave the ferment in gaseous form, but may change its molecular form. For example, P can readily form phosphate minerals, such as struvite ($NH_4MgPO_4 \bullet 6H_2O$, a hydrated combination of ammonium, magnesium, and phosphorus), which 'sink' to the bottom of the fermentation or combine with the undigested organic material (biosol).

Since some N is lost as a gas, this results in relatively higher overall P concentrations (higher N:P) at the end of fermentation, when accounting for both liquid and solids (Massé et al. 2007). The relative concentration

[6] Redox – Refers to chemical processes known as oxidation-reduction reactions. Oxidation occurs when a molecule loses electrons and reduction occurs when a molecule gains electrons. An example is the oxidation of iron resulting in 'rust' when electrons are lost from iron. Another example is when plant biomass burns - the oxygen in air gains electrons and results in a more 'reduced' gas, CO_2. During fermentation processes many redox reactions can occur generally resulting in the net gain of electrons in the mixture resulting in a more reduced chemical state. CH_4 gas is highly reduced, however it leaves the system in anaerobic digesters, effectively removing electrons. By contrast the carbon in CO_2, which may predominate in farm-made ferments, is highly oxidized, and also leaves the system. This abundance of electrons remaining in the ferment influences the chemical form of many compounds.
[7] Solubility - The degree to which a solid or a gas can dissolve in a liquid (e.g. water). The greater the solubility, the greater quantity of a substance can dissolve in the liquid. Substances which do not dissolve easily are 'insoluble'.

of N, P, and other compounds varies based on the starting material, the pH, and the dynamic equilibrium[8] between dissolved forms in the liquid, and solids such as carbonates and phosphates (Möller and Müller 2012). Though these findings derive from research from biogas digesters, they are equally relevant to farm-made biol and central to its use as a tool for soil fertility management in the Andes.

[8] Dynamic equilibrium - The concept describes how chemical reactions are reversible. An example is the reaction of ammonia, NH_3, in H_2O to form ammonium, NH_4^+ $NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$. The reaction is reversible, and the concentration of each compound depends on factors such as temperature and pH.

7. Andean context for the use of liquid ferments and biol

The literature review did not reveal ancient origins of the use of liquid ferment in Andean crops. Along with the use of guano (Cushman 2013), traditional soil fertility management in the Andean altiplano includes the collection and storage of manure from domesticated camelids. Subsequent treatment may include applying manure as a paste to the potato crop at planting time (van den Berg 1989, Garcia et al. 2015).

A. "Biol" in literature

The origin of the term "biol" has not been found, but it appears to correspond with the use of liquid slurry from anaerobic digesters and the rise of fermented preparations used exclusively for crops. (Gomero Osorio 2005). One of the justifications for biogas production was the material inputs (manure and residual biomass), predominantly of local origin and low cost. Similarly, this became the appeal of locally produced fermented fertilizers to augment or displace fertilizers of expensive origin. Figure 11 illustrates the inputs of the different types of biol.

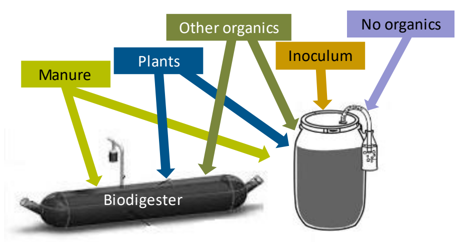


Figure 11: Comparison of inputs to biodigesters and on-farm biol, divided into different categories. Biodigesters can produce biogas from a multitude of organic inputs, but typically a single digester does not have diverse inputs. Generally, biol includes more diverse inputs from numerous categories, including organic and non-organic, and inoculums.

The term "biol" has changed over time and continues to have mixed connotations, as we have observed in the literature and in interviews. In the present study we refer to biol as a liquid fermentation of manure, whether prepared for biogas production or not. For biol made exclusively for addition to crops, we use the term liquid ferment "made on the farm" or the adjective "artisanal".

Research Insight – Inputs to liquid ferments

Farm-made liquid ferments in the Andes are notable for their large diversity of ingredients. However, this characteristic may also be rooted in biogas production. A review of biogas digesters using manures found dozens of other organic inputs, including food scraps, forage plants and residual waste from dairy, fish, fruit and vegetable processing (González and Jurado 2017). In the Andes, camelid and guinea pig manure is high in N content (Alvarez et al. 2006) leading to excess NH_3 which inhibits biogas production (Ferrer et al. 2009). Manure in this region is blended with plant biomass and other waste streams (Alvarez and Lidén 2008a, 2008b) to lower the C:N of the starting material to ranges between 15:1 and 45:1 to optimize digester operation for biogas production (Lansing et al. 2010). This may not be a factor for farm-made ferments - little research exists on the ultimate form and concentration of nutrients based on different feedstocks to optimize farm-made ferments for agroecological application.

Among the literature selected for review, approximately 26 sources describe biogas digester effluent as "biol". Most of this literature has little or no information on digester-specific inputs, focusing instead on the use of biol on crops. In contrast, the literature covering farm-made biol lists the ingredients and describes the preparation. Nearly half of the biol preparations reviewed list bovine manure - from dairy or beef - as the base input for fermentation (Figure 12A). Other manure sources included guinea pig, chicken, sheep, rabbit or camelids. Approximately 12% of the preparations used mixtures of different manures, and some studies explicitly compare the properties of the biol made from different types of manure.

The most common ingredient added to artisanal biol is unrefined sugar, such as molasses (Figure 12B). Other common additions are plant biomass (often leguminous forage species), a dairy input (milk or whey), and wood ash. Approximately 15% of the preparations include some non-organic input, such as rock phosphate, borax, zeolite, magnesium sulfate, copper sulfate and other salts.

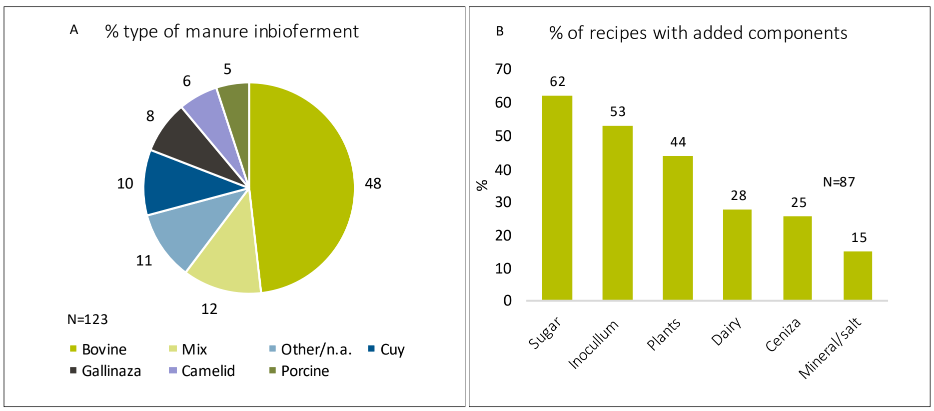


*Figure 12. Distribution of manure types used in 123 biol preparations collected from manuals, academic studies and theses (**A**), and proportion of ingredients of artisanal biol out of 87 described preparations, which included additional components (**B**).*

B. Role of biol components

The literature review included both experimental field trials with biol and promotional material aimed at on-farm preparation and use of liquid ferments. Explanations of the purpose of each ingredient varied according to the source. Manure is the main source of nutrients, such as N, P and K, which are converted into plant-available forms through fermentation. Fermentation of plant material also releases nutrients. Manuals, in particular, also promote the use of plants for their secondary properties, such as insecticidal compounds that can remain bioactive after fermentation. Dairy sources provide both a source of energy (sugars) for the microorganisms and nutrients (N, P, minerals and vitamins). Figure 13 reflects the diversity of organic components found in the literature survey.

Some publications refer to the addition of sugar as a fermentation stimulant. Sugars, accelerate microbial growth, but do not contain substantial plant nutrients. Unrefined sugars contain micronutrients and traces of heavy metals, such as lead, cadmium and arsenic (Quiñones Ramirez et al. 2016). The role of many non-organic minerals is not often described, although salts, such as zinc, iron and copper sulfates, are common micronutrients in foliar fertilizer formulations. (Melendez and Molina 2002). Inputs such as borax and calcium carbonate also act as buffers for the acidity generated during fermentation.

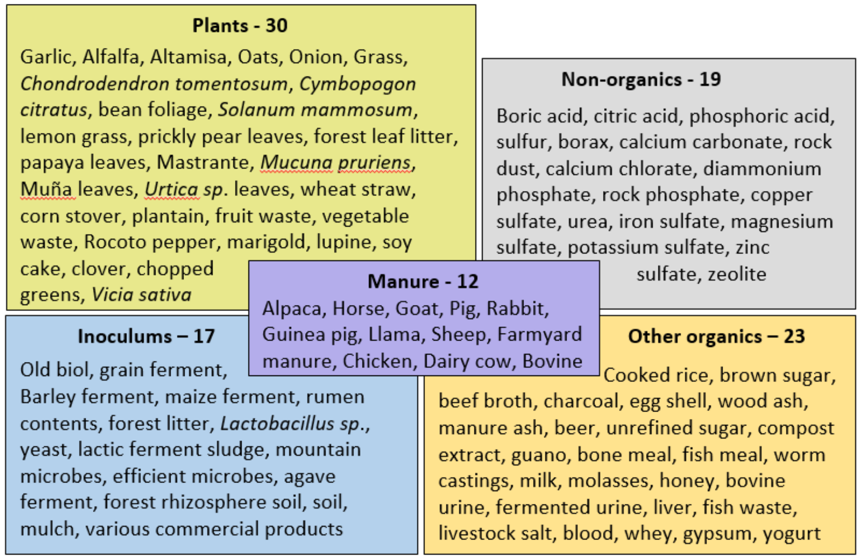


Figure 13: All ingredients added to biol found in the review (research, promotional and academic literature), divided into five categories: plants, manures, other organics, non-organics and inoculums. Numbers refer to the number of distinct inputs dictated within each category.

This study suggests that inoculums, such as yeast, aid the fermentation process, while soil or EM inoculums are intended to provide beneficial microbes for plants. In the case of the latter, the literature states that such inocula share plant growth-promoting bacteria with the biol. Even without inoculation, inputs such as manure and dairy products carry distinct microbial communities that effectively seed fermentations.

Research Insight – Microbial Inoculums

Numerous agronomic benefits are ascribed to microbial inoculums for biol production such as effective microorganisms (EM) (Higa 1994). Reports of positive effects on plants from EM are unclear and inadequate and in some cases benefits derive from nutrients in the inoculum rather than any direct effect of microbes (Mayer et al. 2010, Owen et al. 2015). Microbes with traits for N-fixation or phosphate solubilization are not likely to be favored in manure-based liquid ferments rich in plant nutrients. Indeed, microbes with these traits are isolated and enriched using culture media which does not contain N or soluble P (Pérez et al. 2007, Baldani et al. 2014). Phosphate solubilizing bacteria can have clear benefits on certain soils (Alori, et al. 2017) but can also decline rapidly after introduction to soil (Khan et al. 2007). The benefits of some microbial inocula may ultimately not be practical or cost-effective for small farmers (Aguilar Carpio et al. 2015).

C. Fermentation process and control

Despite the great diversity of ingredients, the basic steps for making biol are similar, whether in a biogas reactor or in an on-farm, artisanal way for crops. This includes nutrient-rich feedstock (e.g., manure), anaerobic conditions and a design that allows gas extraction. Manuals vary in the details of preparing artisanal biol: some simply list inputs and quantities, others add steps such as cutting plants into small pieces to promote decomposition or, in the case of INIAP (Ecuador), have tables with nutrient values of different organic inputs to aid formulation.

Almost all manure-based artisanal biols (described in journals, manuals, academic theses) are anaerobic preparations, with few exceptions (Carrasco Nina et al. 2018). The manuals specify simple vessels for anaerobic conditions, with a valve at the top to allow gas exchange, often through a hose connected to a plastic bottle with a water trap. In some cases, fermentation equipment is equipped with a mechanism to mix the ingredients and accelerate fermentation. The minimum fermentation time indicated for artisanal biol is 30 days, although more than half of the manuals recommend longer fermentation times, up to 120 days. It is recommended that fermentation containers be kept in a shaded places with stable temperatures.

Research Insight – Fermentation conditions
Low temperatures at high altitudes slow fermentation. In the Andes many digesters are built within heat-trapping enclosures (Alvarez and Lidén 2008c). Methanogens require stable temperatures and near neutral pH (Alvarez and Lidén 2008c, Garfí et al. 2016), but in general, optimal fermentation occurs under stable, moderate temeperature. Mixing can promote more complete fermentation by reducing sedimentation (Alvarez and Lidén 2008c, Garfí et al. 2016). Avoiding cold and assuring ingredients are well-mixed (including reducing the size of inputs like plant material so microbes have access to organic material) are equally important factors for farm-made liquid ferment to accelerate fermentation.

D. Manuals and promotion of biol in the Andes

The manuals that promote biogas come from NGOs and government entities (Table 2). Institutions affiliated with biogas promotion, such as USAID, HIVOS and Biosistema, have the least detail on biogas preparation. The Biobolsa manual advocates amending the biogas-derived biol with industrial fertilizers, urea and phosphoric acid, before applying it to crops. Some manuals (CEDEPAS in Peru) promote materials that can be expensive and more difficult to acquire, such as iron sulfate and borax. Most of the manuals (AGRUCO and PROINPA in Bolivia, FOCONDES and ITDG in Peru, FONAG, INIAP and ECHO in Ecuador) promote readily available ingredients, such as ashes, various plants and molasses. Among all the manuals, the percentage of manure to be added to the biol ranges from 3 to 48%, with most manuals recommending approximately 25% manure (with water making up most of the rest) of the total fermentation volume.

Table 2. Selection of manuals from the literature review, with title, affiliation, country of origin, type of biol[9] .

Title	Affiliations	Country	Biol
Production of supermagro	Centro Ecuménico de Promoción y Acción Social (CEDEPAS)	Peru	Artisanal
Preparation and use of biol	Soluciones Prácticas – Intermediate Technology Developing Group (ITDG)	Peru	Artisanal
Biol manual	Biobolsa	Mexico	Biogas
Preparation manual for natural products for soil fertility	Agroecología Universidad Cochabamba (AGRUCO)	Bolivia	Artisanal
Production of natural and ecological, liquid biol fertilizer	Instituto Nacional de Investigación y Extensión Agraria (INIA)	Peru	Artisanal
Production and use of organic fertilizers	Fondo de cooperación para el desarrollo social (FOCONDES)	Peru	Artisanal
Production manual for organic fertilizers	Instituto Nacional de Investigaciones Agropecuarias (INIAP)	Ecuador	Artisanal
Agroecological guide for resilient agriculture	Oficina de Ayuda Humanitaria y Protección Civil de la Comisión Europea (ECHO)	Ecuador	Artisanal
Manual of bio-preparations for ecological agriculture	Ministerio de Agricultura (Minagri)	Chile	Artisanal
Manual for preparation and application of organic fertilizers and pesticides	Fondo para la Protección del Agua (FONAG)	Ecuador	Artisanal
Technical manual: Installation and use of biogas	USAID, CARE	Peru	Biogas
Biol: homemade biofertilizer for ecological production	PROINPA	Bolivia	Artisanal
Biol: the supreme fertilizer	HIVOS people unlimited	n.d.	Biogas

[9] Manual references: Biobolsa n.d., Aliaga n.a., Colque et al. 2005, Alvarez 2010, Chungara Atalaya et al. 2010, Mosquera 2010, Feican Mejia 2011, Infante Lira 2011, FONCODES 2014, Warnars and Oppenoorth 2014, Guzñay D. 2015, Tapia Tapia 2016..

Table 3 reflects how the manuals define the properties and uses of biol. More than 90% of the manuals describe biol as a foliar spray, while about half use the term "biofertilizer" and all apply the term "fertilizer". One third of the manuals describe biol as a product containing plant hormones, half attribute insecticidal properties to it and more than 75% mention efficacy against "pests". All the manuals produced in the Andean region mention the efficacy of biol for plants recovering from frost or hailstorms. Approximately half of the manuals focus exclusively on biosol, and another half include other practices such as the preparation of compost, mineral broths, bokashi or other organic inputs.

Table 3. Selected text from promotional manuals describing the properties of biol.

Affiliations	Biol properties cited in the manual
CEDEPAS	*an aerobic decomposition of diverse organic materials and minerals used as a foliar fertilizer*
ITDG	*And organic, foliar fertilizer,...from the process of anaerobic fermentation of manure and harvest litter*
Biobolsa	*Liquid organic foliar fertilizer from the decomposition of manure, green plants, in the absence of oxygen*
AGRUCO	*...from organic material produced - microbial activity transforms into vitamins, acids, phytohormones and minerals*
INIA	*...anaerobic decomposition...mixture of manure, chopped alfalfa, rock phosphate, milk, fish and others*
FOCONDES	*...organic foliar fertilizer, prepared from fresh manure and other organic materials...where air does not enter*
INIAP	*anaerobic fermentation of plants with strong odor, and chopped legumes mixed with fresh manure*
ECHO	*liquid fertilizer, processes anaerobically...milk, molasses for energy, excrement from cattle, yeast*
Minagri	*An organic foliar fertilizer, product of anaerobic fermentation of organic wastes, manure, harvest residues*
FONAG	*foliar fertilizer...made from different manures that should ferment for two to three months in a plastic barrel*
USAID	*Biol and biosol are natural fertilizers for crops*
PROINPA	*Homemade foliar fertilizer, from anaerobic fermentation of organic wastes of animal and vegetable origin*
HIVOS	*Biol is from animal residues to which urine is added for nitrogen to accelerate the compost process*

The types of manure and the amount added to the biol vary between 3-48% in the manuals describing the preparation, as well as the range of other ingredients (Table 4). Recommended fermentation times range from 30-120 days. In general, the manuals suggest that biol can be used on all types of crops, including vegetables, fruits, cereals and pastures. Most manuals specify a concentration for biol application, ranging from 0.25% to 50% dilution in water (Table 4), but not an application rate, such as volume per field area of a crop. Half of the manuals indicate an application frequency, ranging from every 10-15 days or 2-12 times throughout crop growth, depending on crop type and plant growth stage (Table 4). Nearly half of the guides also mention the value of remaining solids, or biosol, as a soil fertility amendment. Less than half of the manuals list the chemical properties of biosol. Reported concentrations for N, P, Ca, Mg, for example, varied by orders of magnitude among the different manuals, and ranges for pH from 3.59 to 7.5. For further analysis of the chemical and microbiological properties of the biosol we did not use the data from these manuals.

Table 4: Summary of the preparation and use of biol in the promotion manuals. Affiliation corresponds to the institution that prepared the manual (see Table 3). Manure refers to the type of manure and the percentage (%) added to prepare the biol, as well as other ingredients prescribed for the preparation (n.a. if not listed). Days refers to the range of days for fermentation, Conc. refers to the concentration of the biol for field application (% diluted in water), and the frequency of application of the biol (d.= days, q. = every).

Affiliation	Manure	(%)	Other ingredients	Days	Conc.	Freq.
CEDEPAS	n.a.	22	Milk, molasses, zinc sulfate, magnesium, iron, copper, borax, bone meal, blood, liver meal, fish meal	30-45	1-5%	q. 10-15 d.
ITDG	G. pig, bovine	25	Molasses, egg shell, ash, dairy whey, livestock mineral salt, barley ferment	60-90	2.5-10%	n.a.
Biobolsa	Bovine, porcine	n.a.	Urea, diammonium phosphate, citric acid, phosphoric acid	n.a.	0.25-33%	2-12 times
AGRUCO	Cow	25	Alfalfa or bean extract, wood ash	30-60	10-20%	q.10-15 d.
INIA	Sheep, cow, g. pig, chicken	19	Fish, ash, brown sugar, alfalfa, ortiga, cow urine, milk, rock phosphate, barley ferment	30-120	n.a.	n.a.
FOCONDES	Cow, g. pig	27	Legumes (alfalfa, clover) molasses, ashes, dairy whey, grain ferment, yeast	90-120	5-25%	q. 10-15 d.
INIAP	Cow, pig, g. pig, chicken	14	Plants (e.g. altamisa, ortiga,ruda, turpac,alfalfa, poroto), peppers, egg shells, ash, molasses, milk, minerals, yeast	90	n.a.	n.a.
ECHO	Cow	10	Milk, molasses, *mucuna p*. sugar cane leaves, onion, garlic, lemon verbena, mastrante, marigold, ash, rock phosphate, wild microbes, yeast	30	2.5-5%	n.a.
Minagri	Cow	48	Leguminous forage	90+	15-20%	3-5 times
FONAG	G. pig, chicken, cow	3	Molasses, ash, whatever organic waste at hand	n.a.	5-7.5%	n.a.
USAID	n.a.		n.a.	n.a.	2.5-10%	n.a.
PROINPA	Cow, pig, rabbit, other	25	Ash, milk or whey, unrefined sugar, legume leaves, leaves from 'repellent' plants, 'Biograd' inoculum	40-90	0.05	q. 15 d.
HIVOS	n.a.	n.a.	n.a.	n.a.	varies	various

E. Biol preparation and use in the field

Interviews with farmers and promoters were used to summarize knowledge of biol, its preparation and use in the field. All interviewees talk about the biol made on the farm and not the one associated with biogas. Under this approach, the way biols are described and the factors that are considered fundamental for their preparation were captured. Responses were summarized for multiple interviews across regions and countries and, where possible, comparisons were made based on geographic factors such as region and altitude.

Table 5. Summary of biol description, preparation and inputs, from farmers and promoters in Ecuador, Bolivia & Peru

PROVINCE		BIOL & PREPARATION	INGREDIENTS
ECUADOR	AZUAY	**DESCRIPTION**: phytostimulant, biofertilizer, organic fertilizer, foliar biofertilizer, good material. **ELABORATION:** ELABORATION: anaerobic, (two people consider aerobic), consider lunar cycles, addition of minerals, recipient location, mixing 2 times per week.	**IRREPLACEABLES:** non-chlorinated water, manure, raw sugar cane, herbs (chili, garlic, rue, onion, nettle), fruits, chicha, yeast, ammonium molybdate. **PURCHASE:** manure, molasses, milk, minerals, rice powder, sulfates, copper, borax.
	PICHINCHA	**DESCRIPTION**: anaerobic fermentation, cattle manure, ruminant manure, biofertilizer, bioenergizer, manure tea, organic fertilizer, biopreparation. **ELABORATION:** anaerobic, (one people considers aerobic), fresh manure, chlorine-free water.	**IRREPLACEABLES:** manure, molasses, ash, whey, mountain microorganisms, yeast, rock dust, legumes, strong herbs. **PURCHASE**: sulfates**,** molasses, stone dust, minerals, whey (milk), lime.
BOLIVIA	COCHABAMBA	**DESCRIPTION**: biofertilizer, foliar fertilizer, nutrient balancer. **ELABORATION:** anaerobic, (one people considers aerobic), fresh manure and legume chopped, recipient location, harvesting - bubbling in the bottle.	**IRREPLACEABLES:** fresh cow manure, ash and leguminous herbs, chancaca, pure water, alfalfa, no 'unique' recipe. **PURCHASE:** container, milk, chancaca
	LA PAZ	**DESCRIPTION**: biofertilizer, liquid ferment, fermented fertilizer, natural fertilizer, bioinput. **ELABORATION:** anaerobic, (one people considers aerobic), homogeneous mixture, water quality.	**IRREPLACEABLES:** fresh cow manure, chicken manure, chancaca, alfalfa. **PURCHASE:** recipient, milk, sugar, yeast, muña, artemisa.
	ORURO	**DESCRIPTION**: foliar biofertilizer, foliar organic fertilizer, liquid bioinput. **ELABORATION:** anaerobic, homogeneous mixture.	**IRREPLACEABLES:** fresh cow manure, yeast, chancaca, water, muña. **PURCHASE:** milk, sugar, yeast, alfalfa.
PERU	ÁNCASH	**DESCRIPTION**: bioferment, foliar fertilizer, organic fertilizer, biofertilizer, plant food. **ELABORATION:** anaerobic, (one people considers aerobic), keep away from children, temperature control, amount of ingredients.	**IRREPLACEABLES:** manure, molasses, alfalfa, clover, island guano, milk, black earth, ash, phosphate rock, fish offal, EM. **PURCHASE:** yeast, fish offal, island guano, sugar, EM, mineral salts.
	HUÁNUCO	**DESCRIPTION**: organic fertilizer, foliar alternative, phytostimulant, natural chemical. **ELABORATION:** anaerobic, remember times, mix and chopped herbs	**IRREPLACEABLES:** manure, chancaca, herbs, banana peel, alfalfa, fish, mineral zinc, magnesium, boron, ashes, eggshell. **PURCHASE:** recipient, yeast, fish meal, island guano, sugar, EM, minerals.
	LIMA	**DESCRIPTION**: foliar fertilizer, foliar amendment, organic liquid fertilizer. **ELABORATION:** anaerobic, (one people considers aerobic), do not fill the recipient.	**IRREPLACEABLE:** manure, chancaca, herbs, whey, yeast, potassium, phosphorus, EM. **PURCHASE:** yeast, fish offal, blood, manure, milk or whey, lime, EM.

Farmers and promoters described critical aspects of liquid ferment preparation (Table 5). In seven of the eight provinces evaluated in the three countries, at least two dozen variations of terms were collected to describe the biol, such as "biofertilizer," "foliar fertilizer," "phytostimulator," and "liquid bioinput." Almost all mentioned the importance of using fresh manure and keeping the mixture isolated to ensure anaerobic conditions. Recommendations on periodic mixing of ingredients, container location for stable temperature, and safety and the use of non-chlorinated water were also noted. Respondents also considered a source of sugar and plants to be a necessary ingredient, but many other ingredients, such as the inclusion of mineral salts, varied between regions.

The availability and mix of inputs differed markedly among regions. In Peru, the inclusion of fish waste and EM appeared to be different, and the ingredients purchased varied, including manure in the Ecuadorian regions. In the province of Oruro (Bolivia), one farmer stated ***"...we have to buy from La Paz, muña, altamisa, alfalfa, because in the altiplano it is dry. The only thing we get in our area is cow dung and milk whey".*** A farmer from Pichincha (Ecuador) noted, ***"Determining the right amounts of each ingredient in the biol is the most important process to ensure its quality. "***

Farmers interviewed in the field identified empirical indicators of complete fermentation and when a biol is ready for use. These included an alcoholic fermentation aroma, which some referred to as "chicha smell," and a surface color ranging from amber to greenish, but never black. Some reported microbial growth in the form of white specks on the surface as a desirable indicator of advanced fermentation.

Farmers reported fermentation times ranging from 14 to 150 days (Table 6). Little correspondence was observed between altitude (and therefore temperature) and reported fermentation times. In Pichincha, at an altitude of 2,300 meters, fermentation times of 30-90 days were reported, while in Ancash, at 3,150 meters, farmers reported fermentations of 10-15 days and in highland areas of Cochabamba, at 4,200 meters, fermentations of 21 days. In all regions, most interviewees reported ideal fermentation times between 30 and 60 days. However, the great variability in responses to fermentation times does not indicate a clear pattern based on locality or altitude, nor a correspondence with the properties of the final product.

Table 6: Region and altitude in meters above sea level with days of fermentation, concentration (% of biol) and frequency of application, according to testimonies of farmers and promoters.

Province	Altitude (m.a.s.l.)	Fermentation time (days)	% Biol (application)
Azuay	2500-3400	20-80	5% (every 30-60 days)
			5% (1x lettuce - 2x strawberry
			5.5% (every 15-30 days)
			5% foliar o 2.5% soil (n.a.)
Pichincha	1890-3000	14-90	5% (depends on the crop)
			2.5-10% (1x cycle start)
			10% (every 15 days)
			0.2% (every 8 days)
			25% (every 7-15 days)
Cochabamba	2750-4200	21-30	4-80% (2-3 times)
			85% (every 7-14 days)
La Paz	3700	90-150	5-11-1% (every 15 days)
Oruro	3670-3834	30-150	n.a.
Áncash	2160-3860	20-120	5-8.1% (every 7 days)
			0.5%-1% (every 10-12 days)
Huánuco	2000-3250	25-80	5% (every 10 days)
			17.6% (every 15 days)
Lima	2250	30-90	n.a.

Of the 93 promoters and farmers interviewed, only 16 reported the dose and/or frequency of application of the biol; and 38 indicated only the frequency of use (Table 6). Similar to the findings from the literature review, doses and frequencies varied considerably. The interviews reflect that the most common dose in the Andean region is the 5% dilution of biol in water (1:20). There was no specific information on the amount applied per unit area of field. Although most farmers indicate the same dose for all types of crops, in some cases specific doses are used for a particular plant or a particular pest or disease. The most commonly reported frequency of biol application was every 15 to 20 days (Table 6).

8. Properties of biol in the Andes

To evaluate the chemical properties of liquid ferments, we extracted data from research articles and academic theses. Almost all of this research refers to the ferment as "biol," either derived from digested substances from biogas production or from a ferment made on the farm. Similar to the variability of biol inputs in the manuals (Table 5), the theses and research articles also reflect various inputs (Figure 13) and preparations. In the case of research studies that report the amount of initial components and include chemical analyses, we compared the nutrient values of biols.

A. Nutrients in liquid ferments

Nitrogen - To analyze the relationship between the amount of manure added and N in biols, we compared 54 fermenter preparations from eighteen studies that used similar analytical methods and converted all units to milligrams total N per kilogram (mg/Kg) of biol. Higher manure content used in the preparation was associated with higher N concentration after fermentation (Figure 14A). Total N increased with the amount of manure, by approximately 77 mg/Kg for each percentage increase in manure content. However, this relationship was not particularly strong, with a large variation among studies, and concentrations ranging from 132 to 6500 mg/Kg.

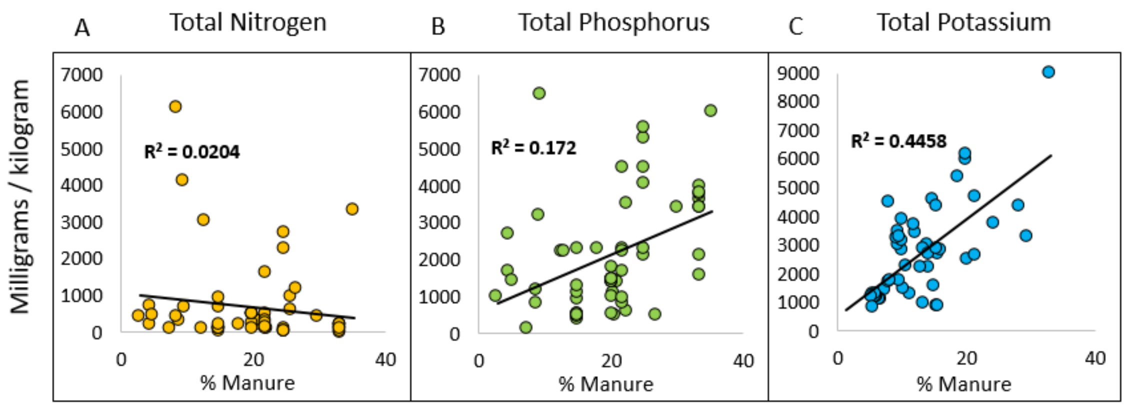


Figure 14. Relationship between nutrient content in parts mg/Kg and manure content as a percentage of total slurry volume. A) N concentration (total N Kjeldahl) of 54 different preparations from 18 studies. B) P concentration of 56 preparations from 20 studies. C) K concentration of 51 different preparations from 15 studies.

Several factors could contribute to this relatively low correlation. First, these comparisons included all types of manure found in the selected sources, although 85% of these studies used bovine manure. The N content of manure varies by animal species and by diet (Möller and Müller 2012). Some studies also included other sources of N, such as dairy products, although these were not added in large amounts.

At least three additional factors explain the variable N content with increasing manure. First, slow or incomplete fermentation could result in lower N release and higher N remaining in the starting material. Second, some of the N may be lost as a gas, such as NH_3 , N_2O or N_2 , during fermentation or storage. Third, N released during fermentation could change form due to pH or other chemical factors and accumulate with the solids in the biosol.

Phosphorus - A comparison of 56 preparations from twenty studies indicates that as more manure is added, the concentration of P in the biol after fermentation remains relatively constant (Figure 14B). Unlike N, the P present in the starting material remains after fermentation. This finding indicates that, in many cases, as more manure is added, there is a limit to the amount of P, released during fermentation, that can dissolve in the liquid ferment, with the remainder as sediment at the bottom or bound to other solids.

A closer examination of specific studies reflects how P concentration can vary among ferments. Ito et al. (2006) compared liquid ferments from twelve different farms, half of which added 1 to 14 kg of rock phosphate. Rock phosphate appeared not to influence P concentration in the biol, and several biols without added phosphate had a higher concentration of P. In contrast, the same study compared several laboratory-prepared fermentations. Those with added dairy whey had the highest P. This further indicates how the starting material and biol chemistry can influence the nutrient concentration in the final ferment.

Other nutrients - In the studies conducted, K shows the strongest relationship between initial manure content and final biol concentration (Figure 14C). Like P, K cannot escape during fermentation, but is more soluble and remains dissolved in the liquid ferment over a range of pH. Variation among studies depends on factors such as manure type, animal diet, and interaction with other ingredients. Many ferments in these studies have added minerals, such as Mg, Ca, copper, boron and manganese, all plant micronutrients used in foliar fertilizers. (Fageria et al. 2009). However, in our study, these micronutrients were also abundant in many biols without these added mineral supplements.

None of the publications reported sulfur content in biols. Sulfur is an important plant nutrient, subject to biological transformation during fermentation, producing hydrogen sulfide gas (H_2S). Documentation of sulfur in manure digestates also seems to be lacking in global studies. (Möller and Müller 2012).

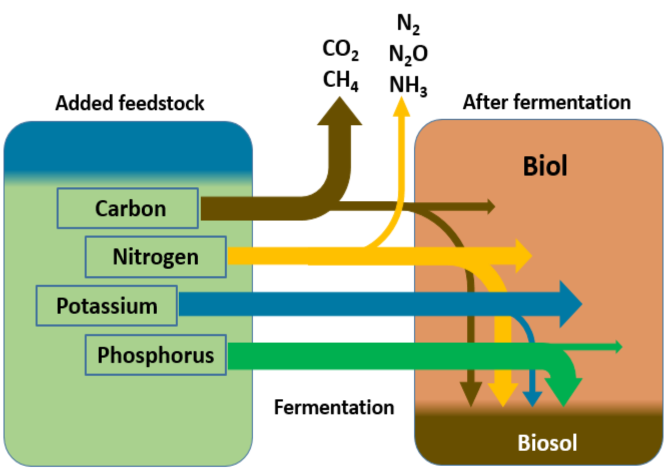


Figure 15. Pathway of key elements in feedstocks – carbon, nitrogen, potassium and phosphorus – over the course of and after fermentation. The width of arrows represents generalized quantities of the fates of each element. Large amounts of carbon leave as gases such as CO_2 and CH_4, some carbon remains diluted and suspended in the biol, and carbon resistant to decomposition forms a sediment biosol. Some nitrogen gas leaves as N_2, N_2O and NH_3, large amounts enter the biol and Biosol. Potassium readily enters the biol. Most phosphorus forms minerals and combines with the Biosol.

A key factor in the calculation of nutrient concentration in the biol, particularly for N and P, is the method in which the ferment sample is taken. This detail is not reported in most of the studies in this review, but may explain the high variation in nutrient concentrations. Standard procedures for the analysis of digestate samples (i.e., the samples in Table 7) require filtering the solids (APHA 1998). The relatively high N concentration and large variability in the biol samples in Figure 14 suggest that the solids were not always filtered, and that some samples contain suspended solids with a high concentration of N. This demonstrates the difficulty of analyzing and comparing between complex preparations. Among the studies reviewed the total solids varied widely due to different amounts of manure and the addition of other ingredients added to the fermentations. Figure 15 reflects a general pathway and fate of key elements in biol.

Nutrients in the anaerobic digestion of the Andes

A review of biogas digesters in the Andes shows the variation in N content in the digestate (Table 7). The percentage of N in the solids from cow manure digestate digestate was 3.05%, and for guinea pig manure it varied between 2.93-5.44% N, which converts to approximately 30,500 and 29,300 - 54,400 mg/Kg, respectively. Total N in the liquid fraction was 271 mg/L in cow digestate and 185-380 mg/L in guinea pig digestate, which is approximately two orders of magnitude less than the percentage of N in the solids. In addition, of the N in the liquid and solid fractions, almost all was in the form of plant-available NH_4^+ (Table 7). These ranges for N in the solid and liquid phases of the digestate are similar to those found in a global review of digestate nutrient content (Nkoa 2014).

Table 7: Chemical parameters of liquid ferments from studies of anaerobic digesters in the Andes (from Garfí et al. 2016), a global review (from Nkoa et al. 2014) and biols from this review. Parameters include percent total solids, percent total N in solids, total N in liquid, ammonia N (NH_4^+) in liquid, P measured as phosphate (P_2O_5) in liquid, and pH.

	Anaerobic digesters		Biol
Parameter	Andes **	Global ***	This revision
Total solids(%)	0.9 - 2.7	1.5 - 45.7	n.d.
N* total - solids (%)	2.9 - 5.4	3.1 - 14.0	n.d.
N* total - liquids (mg/L)	185 - 380	120 - 910	132 - 6500
N-NH_4 (mg/L)	165.5 - 210	150 - 680	114 - 980
P-P_2O_5 (mg/L)	130.2 - 134.5	40 - 260	9 - 423
pH	7.1 - 7.5	7.3 - 9.0	3.7 - 9.2

Total Kjedahl nitrogen. ** from Garfí et al. (2016). * from Nkoa (2014)*

Critical summary – Plant nutrients in biol

Carbon and some nitrogen escape as a gas during fermentation. After fermentation, plant nutrients such as N and P occur in three phases - dissolved in the liquid ferment, in solid sediments (biosol), and in undecomposed solids suspended in the liquid ferment. Among these phases the relative concentration of nutrients such as N and P differs.

On average, manuals in this review recommended manure addition of 25% of the total biol volume. A reasonable N value for fresh bovine manure is 5 grams per kilogram (Bernal et al. 2009). At this concentration, 25 kilograms of fresh bovine manure in a biol volume of 100 liters equates to 125 g manure-N for the entire biol volume – about 1.25 grams per liter of biol. To supply 120 kilos N for one hectare of potatoes would require approximately 1000 liters of biol, and multiple application depending on the dilution rate (typically 1:10). The N in biol will be highly available for plant uptake. However, as Table 7 shows, after fermentation, a large amount of N remains in solids (biosol or suspended), so that the concentration of N in liquid biol may be considerably lower than 1.25 grams per liter.

B. pH of liquid ferments

The pH of liquid ferments is a key indicator of chemical properties: pH drops as acidity increases. When microbes break down sugars into VFA, acidity is generated (i.e., acidogenesis), which lowers the pH. If the pH rises again, this indicates that the VFA are being consumed by the microbes, reducing the acidity.

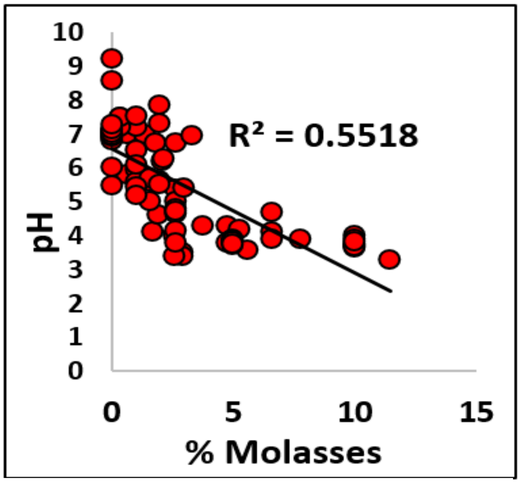


Figure 16: Unrefined sugar (molasses) added to biol as a percent of total volume and pH of liquid ferment. Derived from 86 distinct preparations from 25 published.

In the Andes, we found that most biols are acidic, with an average pH of 5.48 (n=86). For comparison, a review of digestate from biogas plants in the Andes using cow, guinea pig or llama manure had a pH range of 7.10-7.50 among 10 studies. (Garfí et al. 2016) The lower pH of non-biogas biols indicates fermentation with microbial processes other than biogas production.

We found a clear pattern of lower pH (more acidity) with a higher percentage of sugars, such as "molasses," added to the fermentation (Figure 16). This suggests that supplying sugars "up front" accelerates microbial growth and acidity production. In contrast, more complex carbon sources, such as animal manure and plant biomass, have lower sugar content and produce a more gradual fermentation, as hydrolysis releases sugars and VFA are consumed, preventing acid accumulation.

Nitrogen and pH in liquid ferments

During fermentation and anaerobic digestion, up to 50% of manure N is converted to plant-available NH_3 (Bonten et al. 2014). This NH_3 can be lost as a gas at pHs above 7 (Möller and Müller 2012), especially with longer storage and increased handling (Tran et al. 2011). Lower pH reduces N loss.

Critical summary – Adding sugar to liquid ferments

Much of the literature describes sugar addition as a stimulant to fermentation and formation of the biol. Added sugar is an excellent energy source for rapid microbial growth, but may not stimulate breakdown of manure and release of nutrients. For the same studies in figure 14, we found no relationship between sugar addition and N concentration (accounting for % manure), suggesting that added sugar does not stimulate nutrient release from manure.

C. Biosol

Only two studies report chemical analysis of the biosol. In each of them, N concentration was similar in the biol and biosol, but P concentration was substantially higher in the biosol (Aguirre López 2017, Cabos Sánchez et al. 2019). This is in line with observations from a review of anaerobically digested substances in the Andes, where P in the solids of the digested matter is concentrated approximately tenfold relative to the values in the initial manure (Garfí et al. 2016). This results in a higher concentration of P, relative to N (i.e., lower N:P) after digestion, but most of the P is in the solids of the digestate and not in the liquid ferment (Table 7).

Several manuals recognize the nutritive value of biosol as a soil amendment. Others suggest filtering bioliquids through a cloth or sieve before application as a foliar spray. Differences in nutrient properties between liquid biol and biosol, and even fine particles in the liquid, pose a challenge both for standardization and comparison of analyses, and for application of fermentation products to plants.

Research Insight - Solids and nutrient content

The nature of organic matter changes during fermentation. Sugars and other simple energy sources are consumed first, leaving fats and complex fibers, which can enter the liquid fraction (Tambone et al. 2009). This results in fine particles of stable, decomposition-resistant organic material (Tambone et al. 2019) which may contain high concentrations of N and P (Tambone et al. 2017). While the concentration of N may be similar between liquids and solids, the concentration of P is generally several times higher in solids (Kataki 2017). The form of P also depends on pH, with calcium and magnesium phosphates forming as solids as pH increases. (Möller and Müller 2012). Sludge from anaerobic digesters is often treated with acid to "extract" more nutrients from the solids that might otherwise be lost during storage (Möller 2015).

Critical summary – Biosol as a source of plant nutrients and stable organic matter

How a liquid ferment is 'harvested' may determine its agronomic value. For example, if solids are 'resuspened' or filtered out, and whether fine particulates can be applied through foliar sprayers. Biosol has abundant plant nutrients, especially P, as well as stable organic matter. The high N content (as NH_3) in biosol is vulnerable to rapid loss through volatilization (off-gassing) after harvesting biol. 'Rinsing' biosol with additional water may remove more N which can be applied to plants.

D. Microbiology and biochemistry

The literature reporting microbiological data from biols focuses on the presence of common pathogens in manures, such as fecal coliforms (Table 8). Three studies using commercial microbial inoculums reported reduction of fecal coliforms after fermentation compared to the initial manure (Rojas Párraga 2014, Aguirre López 2017, Moreno Ayala 2019). However, fecal coliform reduction also occurred without inoculants, both in the biodigester biol (Soria Fregoso et al. 2001) as well as in the artisanal biol (Ulloa-Cuzco 2015). Garfí et al. (2016) found that higher temperature fermentations reduced fecal coliforms in digesters. Control of human pathogens remains a concern for biol handling and application to crops.

Some studies report the presence of potentially beneficial microorganisms (Ito 2006), such as lactic acid bacteria (LAB), but they also reflect the great variability of microbial communities present in liquid ferments, even among biols from the same study (Robalino Robalino 2011, Villacís-Aldaz et al. 2016). Only one investigation estimated the changes in beneficial bacteria over time, with an increase in population over 121 days of fermentation. (Díaz Montoya 2017). From biogas biol in Peru, Coaguila et al. (2019) isolated N-fixing and nitrifying bacteria. In Costa Rica, Xiu Canche (2018) found low microbial diversity (assessed by molecular techniques) in the preparations of 'wild', mountain microorganisms and in biol itself, and suggested that the growth of some microbes had been inhibited by the added mineral salts.

Table 8: Summary of the results of the microbiological data collected in the literature reviewed. All studies used culture media-based parameters to assess communities. The "(+)" represents positive detection of an organism and "(-)" means no detection.

Microbes detected in biol	Reference
Fecal coliforms: (+) in cuyinaza, (-) in biol (-) biosol.	Aguirre López 2017
Biol from biodigester, decreased total coliforms.	Bastidas 2019
Biodigester: (+) mesophilic bacteria, nitrifying bacteria, actinomycetes.	Coaguila 2018
Mesophilic bacteria increases between 31-121 days in biol.	Díaz Montoya 2017
12 artisanal biols (-) coliforms, (+) *Lactobacillus* sp.	Ito 2006
Fermentation of sheep manure: lowers fecal coliforms to zero.	Medina et al. 2015
Fecal coliforms and *Lactobacillus* sp.: + in manure, less in biol.	Moreno Ayala 2019
Fecal coliforms: (-) in biol.	Peralta-Veran 2016
Fecal coliforms and *Lactobacillus* sp. (+) alpaca feces (-) biol.	Quiñones Ramírez 2016
24 biols with different inoculums (+) *Bacillus* sp. (+ and -) *Lactobacillus* sp. (+ and -) fungi	Robalino Robalino, 2011
The higher the EM dose, the lower the coliform count.	Rojas Parraga 2014
Coliforms (+) in manure, (-) in biol.	Soria Fregoso et al. 2001
Fecal coliforms: (+) in preparation, (-) in biol.	Ulloa-Cuzco 2015
Detected in EM: *Aspergillus* sp., *Rhizopus* sp., *Alternaria* sp., *Penicilium* sp.	Villacís-Aldaz 2016

More than half of the biols studied include some type of inoculum (Tables 4 and 5), ranging from yeasts, to plant and soil ferments, to commercial inoculums such as EM. The impact of inoculum addition is unclear in most studies. Some studies explicitly examined the effect of LAB on lowering the pH of the biol (Quiñones Ramirez et al. 2016). However, we did not find a clear pattern between inoculation and biol pH. Some inoculated biols have a neutral pH and others without inoculation develop an acidic pH. We found no evidence that LAB accelerate the fermentation of complex organic sources such as manure.

Three studies in the Andes estimated plant hormones in the biol, including gibberellic acid (García Fernández 2015)auxins, cytokines (Díaz Montoya 2017) and indolacetic acid (Rojas Párraga 2014) although not all are consistently detected. A study of eight artisanal biols found abundant concentrations of alkaloids, and variable presence of saponins, tannins, and phenolics (Orellana et al. 2013). Studies outside the Andes report that anaerobically digested materials contain vitamins, plant hormones and various other organic compounds, either remnants of introduced material, such as plants, or produced by microorganisms (Möller and Müller 2012, Li et al. 2016). Much of this complexity and its impact on crops and soil remains unexplored in research, and is difficult to separate from the stimulating effect of plant nutrients.

Research Insight – Lactic acid bacteria (LAB)

LAB are used in a number of fermentation applications, and have been proposed as biofertilizer (Lamont et al. 2017). Isolated strains of LAB have been shown to have plant growth promoting properties (Giassi et al. 2016) by acting as biological control agents (Shrestha et al. 2014) and producing antifungal compounds (Gupta and Srivastava 2014). However, LAB are taxonomically diverse and it is unclear whether strains with plant-beneficial traits can grow and multiply in liquid ferments that have manure as their main component. DNA sequencing shows that LAB are a small fraction of the microbial communities in anaerobic manure digests. (Sun et al. 2015).

Urra et al. (2020b) compared a commercial liquid ferment and a liquid ferment made on the farm with molasses, whey, oak litter, wheat bran and basalt dust - neither with manure. Both had an acidic pH between 4.2 and 4.4. DNA sequencing of the commercial product revealed that more than 80% of the microbes were LAB, whereas the farm-made liquid ferment had much higher microbial diversity, with less than 25% of the microbes identified as LAB.

Even if LAB grown in liquid ferments do not have beneficial traits for plants, they can contribute favorable properties to the ferment. Urine fermented with LAB lowers pH, reduces ammonia loss (and thus odor), and improves the value of fertilizer (Andreev et al. 2017). The lactic acid produced by LAB can antagonize pathogens such as *E. coli* and *Salmonella* (Wang et al. 2015) which may be present in liquid ferments.

9. Agronomy and plant growth

A. Effects on plants

Research in the region examines the effect of biol on a range of crops, including corn, quinoa, potato and vegetables such as onion, zucchini and spinach. The studies varied in factors such as the rate and frequency of application of biol, comparison with other fertilizers, and the plant traits measured. Given the variety of biol preparations (e.g., fertilizer types, other ingredients) and the range of crops tested, it was not possible summarize relationships between specific biol properties and crop response.

Ten studies showed no effect of biol on crops and eighteen reported some significant effect. Of these, two reported a negative effect on the crop and 16 reported significant, positive effects on plant traits (Table 9). Sixteen studies exclusively compared biols (e.g., contrasting preparations, or application rates) without comparison to other fertility inputs, and several reported positive effects compared to a water control. Several studies also reported positive effects with higher than recommended concentrations of biol in most promotional materials (i.e., > 30%).

Table 9: Crop plant(s) used in 26 reviewed studies from the Andean region, whether a significant effect was observed on crops, positively or negatively, and a qualitative summary of biol treatment effects.

Crop		Summary of treatment effects	Reference
Phaseolus vulgaris	Si (+)	Biol better than water but not a commercial bioinput	Andino Villafuerte 2011
Solanum tuberosum	Si (+)	14% higher yield c/ biol compared to synthetic fertilizer.	Araque Ipiales 2019
Lactuca sativa L.	Si (+)	Biol at 50% dilution increased yield.	Cardeña Curo 2012
Pisum sativum l.	Si (+)	Better harvest with biol than supermagro or compost tea	Carlos I. & Estrada R. 2019
Zea mays L.	Si (+)	Maize taller with biol 30 days after germination.	Chontal et al. 2019
Allium cepa l.	Si (+)	Biol at 50% dilution higher yield of synthetic fertilizer yield.	Coaguila et al. 2019
Lactuca s. & Brassica o.	Si (+)	Cow manure biol better for crop than pig or guinea pig manure.	Criollo et al. 2011
Plukentenia volubili	Si (+)	Biol increased the number of flowers and fruits.	Diaz et al. 2014
Allium cepa	Si (+)	Higher yield with biol at 20% dilution.	Flores Mamani 2015
Hordeum vulgare	Si (+)	Higher yield with guinea pig biol than sheep, cow manure.	Gomez Huanca 2018
Solanum tuberosum	Si (+)	Biol with sheep manure 12.5% better than cow or rabbit manure.	Guato Guato 2016
Cucurbita pepo L.	Si (+)	Fewer insects and improved yield with biol at 10% dilution.	Lopez Segura 2015
Chenopodium quinoa	Si (+)	Higher yield with biol a 60% dilution.	Mamani R. & Aliaga Z. 2017
Lactuca sativa	Si (+)	Increased yield with biol at 6% dilution applied every 15 days	Pomboza-Tamaquiza et al. 2016
Spinacea oleracea	Si (+)	Increased yield with biol at 40% and 60% dilutions.	Siura et al. 2016
Hordeum vulgare	Si (+)	Increased yield with biol at 50% dilution.	Tumiri 2019
Maize, fava, calabaza	Si (+/-)	Maize yield higher c/ biol than water alone. Beans poor c/ biol.	Ebel 2020
Capsicum asp.	No	No difference in biol dilution as high as 15%.	Alava Mendieta 2015
Allium cepa	No	No effect at biol dilution at 40%.	Bello Moreira 2016
Chenopodium quinoa	No	No yield effect with biol diluted to 50%.	Borda Mercado 2013
Solanum ssp.	No	No yield effect with biol diluted to 50%.	Condori-Mamani et al. 2017
Solanum tuberosum	No	Poor yield in biol compared to treatment with synthetic NPK.	Cutipa Chura 2007
Raphanus sativus	No	No difference in yield with biol use.	Leon Becerra 2018
Maize 'chola'	No	No difference in yield with biol use.	Moreno Ayala 2019
Rosa spp.	No	No effect against nematodes with biol at 3%.	Rosero Chavez 2018
Chenopodium p.	Si (-)	Longer time to maturity with biol use.	Ramírez Ochoa et al. 2016

Few studies reported the frequency of application, but among those that applied biol in 15 day intervals or less, (Criollo et al. 2011, Lopez Segura et al. 2015, Pomboza-Tamaquiza et al. 2016), a positive effect appeared more likely. None of these studies systematically tested increased frequency of biol application or compared this with other approaches to adding plant nutrients over the course of plant growth. On the other hand, numerous manuals claimed benefits from adding biol after episodic stress, such as freezing temperatures – no studies tested biol addition on plants after abiotic stress. High biol concentrations (40% - 60% in water) positviely impacted plant growth (Siura et al. 2016, Mamani Reynoso and Aliaga Zeballos 2017, Coaguila et al. 2019, Tumiri 2019). Pomboza-Tamaquiza et. al. (2016) reported significant effects on crop growth at 6% biol in water though their biol contained uniquely high nutrient inputs, including 20% raw milk and 5% bone meal.

At least two studies applied supplemental chemical fertilizer by comparing different doses of biol, in onion (*Allium cepa* l.) and quinoa (*Chenopodium quinoa* willd) and none showed a significant effect of biol on plant traits (Borda Mercado 2013, Bello Moreira et al. 2016). This suggests a reduced impact of biol when nutrients are not limiting, and that other benefits (i.e. plant hormones) may be less evident. Three studies found that high concentrations of biol reduced seed germination, possibly due to low pH or high salts present in the biol. (Medina et al. 2015, Quiñones Ramirez et al. 2016, Aguirre López 2017). Two studies examined plant pests, on *Cucurbita pepo* L. where biol reduced insect pressure and on Rosa sp. where biol reduced insect pressure. (Lopez Segura et al. 2015) and on *Rosa sp.* where the biol showed no significant impact on a number of insect and fungal pests. (Rosero Chávez 2018).

A single study controlled for total N input, at 150 Kg per hectare, in testing the response of potato (*Solanum tuberosum*) to industrial fertilizer compared to commercial compost supplemented with biol (Araque Ipiales 2019). Compost was applied at a rate of just over 130 Kg N per hectare and an additional 20 applications of biol totalling just over ~20 Kg N per hectare. At each application most of the biol was added as a soil drench at a 50% dilution, and some applied as a foliar spray diluted to 10% in water. For treatments with industrial fertilizer, urea-N was split into two application during plant growth, and 300 Kg per hectare P and 100 Kg per hectare K were added at planting. Numerous additional inputs were applied to control for pests across all treatments. The potato yield was 14% higher in treatments with compost and biol compared to industrial fertilizer. No treatment included compost alone, to test for the sole effect of the commercial

Research Insight – Foliar fertilization

Evidence suggests that foliar application of macro and micro nutrients can be rapidly adsorbed by leaves when in soluble form and sometimes when chelated, particularly when plants are visibly stressed from nutrient deficiency (Fageria et al. 2009). Foliar application of protein hydrolysates (plant extracts) for example, can benefit crop growth and yield (Colla et al. 2017) both through a direct fertilization effect and by stimulating physiological pathways in crop plants that respond to plant stress (Ertani et al. 2017). Research suggests manure digests have some properties as fungicides and nematocides (Groot and Bogdanski 2013). Digests of manure, such as biol also contain plant bioactive compounds from microbes (Li et al. 2018) and especially diverse organic compounds from plants, including hormones (He et al. 2022), however these compounds can decline rapidly with longer storage (Li et al. 2016b).

Critical summary – Biol can improve crop growth depending on how and when it is applied

Though highly variable, the review suggests biol can improve crop growth especially when applied at higher concentrations, or with greater frequency. No studies report effects of biol after physical damage such as freezing, however as foliar fertilizers, they may relieve abiotic stress.

compost, however the results indicates the compost + biol treatment has a yield effect beyond those provided by optimizing macronutrients from industrial fertilizer.

A comparison of commercial liquid ferment, farm-made ferment, and mineral NPK on corn yield showed no difference after one season, and significantly higher yield with NPK in the second year, with the farm-made amendment having the lowest yield (Urra et al. 2020b). A parallel study found a significant improvement in lettuce yield with liquid ferments compared to the addition of industrial NPK. (Urra et al. 2020a). A comparison of organic fertilizers (including supermagro) showed no benefit to corn yield and no difference when used in conjunction with industrial fertilizer supplementation applied at a 50% rate (Vázquez Gálvez et al. 2014). These findings indicate the effect of liquid ferments may depend on crop type, site and soil interactions for a given comparison with other crop fertility managements.

B. Liquid ferments and soil

Numerous studies in the Andes report routine soil test results from field trials, but not before and after application of biol. A single study in Costa Rica on soils planted to banana found no difference in soil nutrients after biol application but an increase in soil respirations and soil microbial biomass (Ortega Bonilla 2013). Given the dilute biol concentrations applied to a large soil volume, a detectable change in soil nutrients is unliklely, so it is notable that changes in the soil ecosystem response, such as soil respiration, were detected. It is also noteworthy that so few studies measured nutrient properties in biol used in studies

Research Insight – Liquid ferments and soil

Applying liquid ferments to crops increases soil N availability but can lead to N loss at high application rates as soluble N exceeds plant demand (Rigby and Smith 2013). Excess N can be lost to the atmosphere or via run-off. Through digestion, the concentration of P increases even more than N, but predominates in solids (biosol), so plants take up more N from newly applied ferments relative to P (Hao et al. 2016). Many studies compare soil-applied anaerobic digest slurry with mineral fertilizers with some showing superior crop yield with the latter (Lee et al. 2020) and others with no difference in yield between the two (Ren et al. 2020). Variability in crop response may depend on whether comparisons are made on the basis of total N or plant available N in liquid ferments. Even without detectable changes in soil nutrients, numerous studies show that liquid ferments stimulate soil ecosystem processes such as soil microbial respiration and N mineralization rates (Möller and Müller 2012).

Microbes in liquid ferments drive biochemical transformations, but appear not to persist once applied to soil. Numerous studies report significant effects on plant growth and soil properties following application of liquid ferments without significant changes to the soil microbial community (Sapp et al. 2015, Coelho et al. 2020, Urra et al. 2020b). Even though composition of the soil microbial community did not change, Alburquerque et al. (2012) found liquid ferments increase soil microbial biomass and enzyme activity. Insam et. al. (2015) also found that manure digestate reduced soil pathogens.

A study comparing commercial liquid ferment and farm-made ferment, found both significantly improved soil properties (respiration, N availability and soil enzyme activity) compared to application of industrial NPK (Urra et al. 2020b). The effect of ferments on soil organic matter (SOM) depends on the types of inputs used ot make liquid ferments. Inputs which are easily degradable, such as legume biomass appear to produce digest which contributes little to SOM, while inputs such as corn stover can result in ferments which build SOM (Möller 2015). It should be noted that few studies make distinctions between liquid and solid portions of the ferment, which we note above have different nutrient and carbon contents. As with variation in crop type, impacts of liquid ferments may vary based on soil type and management history (Urra et al. 2020a), as well as how the ferment is applied.

together with soil testing before and after application, which may indicate the high cost of analytical testing, especially over longer term research, and perhaps uncertainty about which metrics to select for testing. Soil health tests which measure a range of soil ecosystem factors may be most suitable for monitoring response to bioinputs such as biol.

Critical summary – Biol and biosol can improve measures of soil health
Liquid ferments can improve soil ecosystem function over the short term, including N availability, soil respiration, soil carbon turnover, soil aggregation and water storage. Soil health impacts can be measured on farm using standard measures (O'Neill et al. 2021) and adapted to local conditions such as soil P availability in Andes soils for which biosol application may be appropriate.

C. Knowledge and use of biol in the Andes

Agroecological practices in the Andes include a variety of crop and nutrient management strategies (Chilon Camacho 2011, Jacobsen et al. 2014). The complexity of the preparation and application of liquid ferments can impact the adoption of this practice by farmers. The use of biol from biodigesters versus the preparation and use of artisanal biol may differ in terms of practical approaches to nutrient management. Through literature and interviews, we examined the status of biol use by smallholder farmers in the Andes.

In interviewing farmers who participated in an agroecological NGO intervention in central Ecuador, Soto (2010) observed that participants adopted the language of agroecology, but perhaps not the practices themselves. Among farmers, knowledge of the use of biol was limited, even if its usefulness in the framework of agroecological practices was accepted. Rios (2009) reported on a farmer in Ecuador who used biol to control pests in potatoes, instead of chemicals. This required more frequent visits to the field to apply the biol, but also resulted in plants with greater vigor and resistance to frost. However, yields were not comparable to those of crops treated with chemical fertilizers, but the cost of inputs was lower. This outcome precipitated development of a women's group in the community that prepares biol collectively, which was commercialized as an income source. In general, use of biol is widely promoted by NGOs and donors as an agroecological practice. That knowledge of biol preparation and use is not as widespread as awareness of its potential value further suggests use of biol is both introduced and is not spread via grassroots farmer movements.

The relative complexity of biol use – acquiring equipment and inputs, preparing suitable and timley fermentions, applying in a agronomically effective way in concert with other practices - may lend itself to collective preparation (e.g. among a women's cooperative as above) and a division in practices between promoter, preparer and user. This complexity may also explain why compost use was widespread among farmers interviewed in Huancayo, Peru, whereas biol use was more scattered among specific farmers (Chávez Soto and Elescano Lopez 2017). Biol requires greater knowledge, time, and perhaps economies of scale that can accommodate the formulation, preparation and application of biol.

Acrosss the Junin region, Cóndor Quispe (2010) notes the importance of farmer participation in training courses – further indicating biol as an introduced practice. Farmers who attended repeated trainings possessed a deeper understanding of holistic practices and biol use compared to occasional attendees. Participatory activities among farmers in trainings also distinguished producers who ultimately used biol technology, and increased the exchange of knowledge between farmers and promotors. This accords with findings that promotion of complex practices such as soil conservation in the Andes is most effective with a mix of sufficient technical support from outside as well as participatory practices among farmers (Posthumus et al. 2010).

The Yapuchiri (traditional 'leader' farmers) in Bolivia characterize biol use as a specialized practice built over time. One producer reports that his regular preparation and use of biol took dedication, but is now common enough that he always maintains a supply (Pardo Valenzuela and Caballero Espinoza 2018). Community members who are not Yapuchiris seek him out for access to biol. Neighbors who initially expressed skepticism observed positive results when biol is prepared and applied correctly to crops. Interviewees noted the value in producing biol instead of traveling to purchase farm inputs. Some also noted that other farmers attempted to copy biol preparation and sell formulations, but this was not successful.

We found little literature on the actual practice of biol use by farmers in the Andes. A study on organic production in coastal Ecuador using biol may be relevant for other regions. (Santos 2011). In the transition to organic practices in cacao (*Theobroma cacao*), knowledge transfer through extension - regular workshops and field trainings - was critical to translate technical information on biol into on-farm use. When preparing the biol, farmers tended to work collectively, in large groups or in pairs, which improved adoption and application of the biol. Prior to cocoa production rehabilitation, 87% of farms did not use any foliar sprays, and after dedicated extension efforts, 92% of farmers use biol.

Critical summary – Preparation and use of farm-made biol vs. biol from biogas digesters

Farm-made biol preparation and use may depend on training and participatory frameworks. Biol derived from biogas is distinct in that the preparation, availability and agronomic value are dictated primarily by optimization of biogas production. Member organizations of RedBioLAC, who develop household anaerobic digesters throughout Latin America, have made biol central to promotion and adoption, as a valuable fertilizer with potential as a marketable source of revenue (Garwood 2010) that may offset the cost of installing a household anaerobic digester (Arrieta Palacios 2016). Programs supporting farm-made biol as an agroecological practice face a distinct set of challenges compared to promotion of biol derived from household biodigesters.

Interviews in Bolivia, Ecuador and Peru reflect a wide range of perceptions about the uses and impacts of biol on the plant and soil. In Cochabamba, farmers and promoters stated that biol helps crop development by providing minerals and plant hormones, and promotes flowering in potato, maize, quinoa, tarwi and other plants. In contrast, a PROINPA promoter stated that in many cases biol is only useful in early crop development. In all three countries surveyed, interviewees stated that biol improves plant development and gives the impression of increased plant vigor (Table 10).

Table 10. Perceptions of farmers and promoters in Ecuador, Bolivia and Peru on the use and impact of biol.

PROVINCE		USES	PERCEIVED IMPACTS
ECUADOR	AZUAY	biostimulant - soil and microbiota fertilizer - repellent - improves quality and yield - nutrient uptake	improved soil (microbial life) - more vigorous plant - recovery and proliferation of biodiversity - cares for the environment
	PICHINCHA	soil and microbiota recovery - corrects deficiencies - stimulates germination - promotes crops and fruit - pest repellent	improved soil and production - increased vigor - increased production and economic income - improved health and nutrition - reduced dependence on pesticide trade
BOLIVIA	COCHABAMBA	improve the development of plants seedbeds, early stage flowers, vegetables, potatoes, maize, quinoa, tarwi - recover crops from frost and hailstorms	increased plant vigor (increased foliage) - increased plant growth - increased production - recovery and proliferation of biodiversity
	LA PAZ	promoting plant development - providing minerals - recovering crops from frost and hailstorms	increased plant vigor - increased yield (relative to those who use agrochemicals) - increased resistance to frost and hailstorms
	ORURO	foliar fertilizer, use on all types of crops, - improve soil structure - strengthen plant (defenses) - animal repellent - recover crops from hailstorms and frost	improved soil - environmental care - increased plant vigor (foliage) - increased resistance to pests (better grain response) - increased yields
PERU	ÁNCASH	foliar fertilizer - develops plant and soil - higher yield - insecticide, fungicide - recovers crop from frosts	improved soil - increased plant vigor (recovery from wilt) - increased production - biodiversity recovery - improved crop (more flowers, more leaves, more buds) - reduced incidence of disease
	HUÁNUCO	foliar fertilizer - promotes soil microorganisms - plant development - pesticide	improved soil - increased plant vigor and production (fruit) - allows continuous production of vegetables
	LIMA	phytostimulant - nitrogen supply - correcting deficiencies - repellent - plant development	improved soil - increased plant vigor - quality of harvested products - increased yields

Especially in Bolivia, farmers highlighted the use of biol on plants after frost or hailstorms. A farmer from the community of Japo Kasa, ayllu Majasaya, stated ***"I have used it for the frost, for the hailstorm it has been good. For the hailstorm, it is necessary to spay".*** Another farmer, who works with PROSUCO in the community of Suramaya, municipality of Caquiaviri, said: ***"Once hail or frost falls, you have to spray immediately, so that the plant recovers quickly. In my opinion, it is as if I were spraying with fertilizer".*** And a field promoter in Salinas Garci de Mendoza: ***"...the recovery of the plant after the frost helps it by 80%, on the other hand, some producers use it to recover after a hailstorm attack".***

Interviewees also report using biol as a pesticide and to improve soil quality (Table 10). In Bolivia, a farmer from Puqui states that the biol ***"...makes the soil more fertile for the plant, that it has nutrients, so that the plant is more resistant and has better growth".*** In Ecuador, a farmer from Guaraquí says of biol: "***by increasing the life of the soil, it increases the retention of humidity and therefore the frequency of irrigation decreases, the plant is more vigorous and is not so much affected by the attack of pests and diseases. And in terms of production, harvests are better, larger, more vigorous and in greater quantity".*** Many of the perceived impacts are difficult to discern, such as increased biodiversity or ecosystem improvement. Noting that biol can be considered a panacea, a HEIFER promoter in Azuay, Ecuador, stated ***"... some farmers, when observing the results in the field, tend to overestimate the effect of biols and stop working other tools that complement the ecological production system..."***. The literature review suggests, at best, biol can complement other agroecological techniques, and a risk exists for conflating benefits of biol with other techniques or bioinputs. As demonstrated above biol may contribute important plant nutrients, but not enough nutrients to sustain crop yields, without other practices such as compost use.

D. Additional bioinputs in the Andes

Our review found other innovative bioinputs in the Andean region. These includes novel use of traditional organic inputs such as camelid manure and lupines for better nutrient management, (Chilon Camacho 2011) and isolation of soil microbes with potential as biofertilizers (Franco et al. 2011). Use of liquid ferments for nutrient management may be used in concert with these developing practices. The extent to which biol use suits agroecosystem management may depaned on available resources such as manure and residues, complementarity with other practices, time constraints faced by farmers, and availability of broader support mechanisms to support use of biol.

Andean soils contain cold-adapted microbes with plant growth promoting properties (Yarzábal and Chica 2017, Pandey and Yarzábal 2019). Free-living nitrogen fixing bacteria associated with quinoa have been isolated (Choque Estrada 2017). From potatoes, bacteria have been isolated that reduce pest pressure and enhance tuber growth (Oswald et al. 2010) and fungal isolates with phosphorus solubilizing capability (Pineda 2014). Native bacterial and fungal isolates, grown in large batch cultures and applied in foliar sprays, increase yield in quinoa (Ortuño et al. 2013). Secondary metabolites extracted from native *Trichoderma sp.* isolates also increase grain yield in quinoa (Ortuño et al. 2017). Benficial microbes have also been collected through farmer networks by isolating fungi and bacterial from farm composts (Franco et al. 2011). These all hold potential as biofertilizers for Andean crops, separate from biol use.

Numerous NGOs and private or semi-private organizations have an important influence on the use of bio-inputs in the Andes. In Bolivia alone bio-inputs are promoted or produced commercially by organizations such as PROSUCO, AGRECOL, PROINPA and PROBIOMA. For example, PROINPA, sells liquid formulations of specific biofertilizer (i.e. plant growth promoting fungi), as well as mixtures of EM, and insecticides with humic acid extracts and trace metals. The efficacy of these bio-inputs on crops may vary based on who produces them and whether they are satisfactorily tested. With the increasing cost of synthetic fertilizers, and millions of dollars in investments in domestic bio-input production facilities in the Andes (Prensa Latina 2022), the influence of commercial bio-inputs is likely to grow significantly. Whether commercial or produced on-farm, bio-inputs may be derived from diverse sources (manure vs. plant extracts) and be used for distinct outcomes (soil fertility or pest management), so potential benefits and risks to their use depends on the specifc amendment and its use (Urra et al. 2019).

The use of biol can be integrated with innovative applications of traditional nutrient resources to improve soil fertility (Chilon Camacho 2011, Garcia et al. 2015). These include the incorporation of urine nutrients with compost for potatoes (Condori-Guarachi et al. 2018) or collective approaches to compost management and use through farmer networks. (Mamani Falcon 2018). A survey of farmers using biosol in Costa Rica revealed that all used at least one other fertility amendment and often several inputs. (Xiu Canche 2018). The use of biol in Andean agroecological systems will require careful evaluation of how this practice compares with other crop and soil management approaches, and the possible trade-offs in time and materials. For example, farmers in Argentina transitioning to agroecological management of *Amaranthus cruentus L.* found that effluent from a simple biodigester had a greater positive impact on yield than application of vermicompost, but yields were not as great as with application of fungal biofertilizer from *Trichoderma sp.* (Cabanillas et al. 2017).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10. The experience of farmers and promoters across the Andes

Interviews with farmers and field promoters were used to summarize learning and training experiences on the use of biol, as well as future perspectives regarding this practice, and to evaluate what factors favor or do not favor the use of biol. This survey included people who have experimented with biol but have stopped using it. With more than 100 interviews conducted, an attempt was made to collect the most repeated and representative statements of the range of opinions from many sources among eight Andean provinces of Bolivia, Ecuador and Peru.

A. Knowledge transmission and learning spaces

Testimonies were collected to understand the methods and tools used to transmit knowledge on the use of biol, as well as the limitations in the learning process. Table 11 reflects a summary of the responses in all provinces evaluated. It should be noted that the comments include both the experiences of the interviewees and the biases that reflect perceptions of learning and training for the use of biol.

In all regions, interviewees cited demonstration of results in the field as key to communicating results. Dialogue of knowledge, sharing of experiences and "farmer-to-farmer" approaches are useful bridges for the spread of the use of biol and other bioinputs. A case in point is that of the Yapuchiri, or wise farmers, in the Bolivian altiplano. The interviewees pointed out that their participation makes teaching and field practice activities more effective. In the provinces studied, the testimonies show the scarce participation of local authorities and governmental entities in the promotion of the use of biol and other related practices. In most cases it has been NGOs that have assumed this role. The weakness of these interventions are their short duration which tend to underscore the way that NGOs depend on donors to stay in an area and their proclivity for changing their focus of intervention too frequently to allow for sustained promotion, farmer learning, and adaptation of practices such as bioles to the local context.

Interviews reflect farmer experiences with biol, but also prevailing attitudes of farmers and promotors, both positive and negative, regarding biol use. Lack of interest, envy, resistence to change, lack of knowledge or belief, and passivity were all given as reasons why farmers do not use biol. A promoter of the BIOTOP biofactory in Bolivia comments: ***"... the farmer, as a farmer, does not yet assume what is organic, for him, the easiest thing he needs, and chemical products are easy to use".*** An agricultural specialist from Pichincha said "... ***people do not understand the basic principles for the production of biols, which has hindered their adoption in the field"***. These comments may reflect latent attitudes of promotors towards farmer practices, or even reveal perceived knowledge capital about biol as a specialized practice, rather than underlying motivations of why farmers use biol or not.

Table 11. Summary of reflections from interviews on biol use learning methods and challenges, recorded by promoters and farmers in Bolivia, Ecuador and Peru.

PROVINCE		COMMENTS ON METHODS AND LEARNING SPACES ON BIOL
ECUADOR	AZUAY	**Methods:** Long-term training, dialogue of knowledge, farmer to farmer, sharing of experiences and group practice, workshops for those interested, exchange of experiences among producers, talks, consultations via WhatsApp, field demonstrations.
		Difficulties: Lack of time and money, lack of interest, resistance to new knowledge, lack of patience, risk due to the transition to agroecology (assuming the losses involved), passive attitude of producers, large groups, lack of information, no difficulty.
	PICHINCHA	**Methods:** Focused on recipes, theoretical and practical workshops (including negative effects of pesticides on human health, and benefits of bioinputs), field visits, agroecology courses, permaculture, etc.
		Difficulties: Theoretical issues, lack of time, lack of interest, requires detailed explanation of processes and demonstration of results in plots, access to materials, resistance to new knowledge, bad experiences in training, lack of understanding of the principles, habit of using agrochemicals, no difficulty.
BOLIVIA	COCHABAMBA	**Methods:** Farmer to farmer, group practice followed by individual practice, preparation and application of biols, information on negative effects of agrochemicals, theoretical and practical workshops with guidance, Yapuchiri training with demonstration plot, non-specific.
		Difficulties: Lack of patience, lack of seeing results in the field, lack of knowledge of agroforestry, understanding of biol (it is not a panacea), lack of interest, envy, resistance to new knowledge, lack of guidance, bad previous experiences, superficial approach.
	LA PAZ	**Methods:** Demonstration plots, exchange of experiences, farmer to farmer, literature review, training with Yapuchiris, theoretical and practical training, non-specific.
		Difficulties: Understanding of the biol (it is not a pesticide), lack of interest, limited accessibility to ingredients, limited access to communities, lack of project follow-up, envy, lack of dedication, long fermentation time.
	ORURO	**Methods:** Farmer to farmer, exchange of theoretical and practical experiences, Yapuchiri trains with demonstration plots, support in audiovisual materials and digital media, self-taught, advice from technicians and agronomists.
		Difficulties: Habit of using agrochemicals, resistance to new knowledge, no belief in positive impacts of biol, high rotation of authorities in communities, lack of interest, long fermentation time.
PERÚ	ÁNCASH	**Methods:** Demonstrative workshop, use of flyers, personalized technical assistance, farmer to farmer, recipe distribution, visits to plots, talks at assemblies, videos, non-specific.
		Difficulties: Lack of awareness, envy (training a small population), no belief in positive impacts of biol, limited access to communities, lack of field demonstrations, bad experiences with technicians, very large groups, local language (Quechua), habit of using agrochemicals.
	HUÁNUCO	**Methods:** Farmer-to-farmer, demonstration plots, demonstration workshop, exchange of experiences, technical personnel who stay 2-3 months, work on organizational strengthening, supply of products or inputs, harvesting.
		Difficulties: Disinterest, envy, lack of belief in the positive impact of biol, habit of using agrochemicals, lack of time, limited access to communities, lack of knowledge and guidance.
	LIMA	**Methods:** Training, recipes and demonstration classes for groups of young people, teachers and community organizations, group practices and then individual practice, exchange of experiences between producer organizations, non-specific.
		Difficulties: Lack of time, habit of using agrochemicals, search for faster results, lack of awareness, lack of interest, non-specific.

B. Factors favoring or disfavoring the use of biol

To understand the adoption and sustainability of biol use by farmers, three factors were assessed: 1) perceived benefits, 2) future perspectives regarding the use of liquid ferments, 3) economic, social and ecological aspects favoring biol use versus discontinued use. The summaries of responses reflect specific observations, beliefs and aspirations. Farmers' reports of the benefits of biol use generally converged across the eight provinces in the study, in order of priority: improved crop quality and yield, leading to higher incomes; savings in the purchase of inputs and agrochemicals, increased autonomy (i.e., reduced dependence on agrochemicals); improved soil quality and fertility; reduced incidence of pests and diseases and associated costs; improved health in terms of nutritious food; care for the environment, biodiversity recovery and ecosystem restoration (Table 12).

Table 12. Perceived benefits and future prospects in relation to the use of biol, according to statements by farmers and promoters in Bolivia, Ecuador and Peru.

COUNTRY	PERCEIVED BENEFITS	FUTURE PERSPECTIVE
ECUADOR	-Economic (income/savings) -Improved health, soil and environment -Product quality -Lower incidence of pests -Autonomy and empowerment (reduced dependence on agrochemicals, farmer status) -Seed care.	-An organic transition, due to the economic and environmental crisis -Recovering the soil -Maintaining knowledge exchange -Public and private sectors promote organic production with subsidies, marketing of agroecological products -Consumers demand healthy and nutritious food -More sales of bioinputs -Breaking dependence on agrochemicals -More economic income and prestige for knowledge.
BOLIVIA	-Better yield, product quality -Economic (income/savings) -Improved soil, lower incidence of pests -Healthy food access -Autonomy and empowerment (Yapuchiris) -Ease of crop management. -Recovery of local knowledge.	-Biol as part of an organic transition -Aware consumers and accessible organic products -Better techniques for the use of biols -Producer organizations promote bioinput -More emphasis on livestock -More economic income, guaranteed sales space, knowledge about biols is passed on to young people -Incentives for organic production -More awareness of environmental care -Harvest of 43 quintals per hectare achieved -Improved biols, wide dissemination among quinoa producers' associations, bioinput marketing opportunities.
PERU	-Better health with healthy food -Exchange of experiences -Economic (income/savings) -Better soil quality -Environmental care -Social cohesion.	-Consumer demand for organic products -Recovery of ancestral practices -Develop better biols and promote their use -Contribute to consumer health -More farmers are encouraged by agroecological production, public sector supports the transition -Public sector prioritizes health and prohibits food consumption with agrochemicals -More opportunities to commercialize bioinputs -More widespread use of biols -Commercialize bioinputs -Continue use and experimentation.

In terms of future prospects, farmers' aspirations reflect that many of those interviewed are in the process of transitioning to agroecology (Table 12). They believe that the growth of agroecological production and market access will improve their quality of life, but only if consumers value agroecological products. In La Paz, Azuay and Pichincha, farmers mentioned that the use of bioinputs should be encouraged by state and private entities through incentives to promote the transition to agroecology. In Azuay, farmers mention a desire to transition to 100% agroecological livestock production. In Huánuco, farmers state that agroecology will only be viable if the State limits agrochemicals instead of promoting their use. In Cochabamba, farmers mention that training in schools will help create awareness and responsibility in caring for the environment. While many of these farmers and promoters express the benefits of using biol, much of their future prospects are tied to factors that are aspirational or not under their control.

When asked to reflect on the factors that favor or disfavor the use of biol, farmers reiterated many of the above reasons, but also revealed deep-rooted views on farming practices and socioeconomic context (Table 13). Visible results in the field are a primary factor favoring the use of biol, as well as the lower cost of production by utilizing more available resources in farm production. However, producers also expressed the benefits of working in groups as an aspect favoring the use of biol - this seems to acknowledge the support of NGOs, farmer groups and social cohesion and associated organization as much as any technical support related to the use of biol. In fact, farmers in Azuay stated that they perceived social resistance to agroecological movements prevents spreading these practices, indicated the influence of movement rather than specific practices.

Farmers who discontinue biol use point to lack of interest and time (Table 13). A large number of promotors ascribed these to attitudinal factors such as lack of knowledge of the negative health and environmental effects of agrochemicals and perception of profits over sustainability or resistance to new knowledge and practices is observed. However, these views are also expressed by some who indicated that training in the field was poor or inadequate (Table 11). Poor training in the field could at least lead to mismatched expectations between promotors and producers. Several interviewees express the often complicated production process as dissuading biol use, especially given insufficient time, technical support or economies of scale for either securing biol inputs or markets for value-added (agroecologically produced) crops.

Table 13. Summary of factors favoring and not favoring the use of biol declared by farmers and promoters in Bolivia, Ecuador and Peru.

COUNTRY	FAVOR THE PRACTICE	DOES NOT FAVOR THE PRACTICE
ECUADOR	-Access to healthy and nutritious food -No agrochemicals, improved quality of life, lower incidence of pests, higher income -Allows crop diversity -Empowerment of farmers -Reuse of farm waste -Interventions by NGOs and some government programs, which have laid the foundations for agroecological development -Socio-political resistance of agroecological movements and better commercialization -Low cost of the process.	-Inadequate training, lack of understanding of the processes -Difficulty with organic certification -Belief that the biol solves all crop problems -Availability of materials (recipient) -Working with manure, blood or animal rumen is considered dirty -Lack of time, work falls on women -Competition exercised by agribusiness -Unemployment and migration to the city -Belief that agroecology demands a lot of effort -Public policies that promote agrochemicals -In Azuay, most producers are women over 50 years of age -Rigid idiosyncrasy and devaluation -Consumers do not value agroecological production.
BOLIVIA	Results in sight -Low cost of the process -Nutritious food that improves health and the environment -Use of local resources -Preventive against pests -Community cohesion -Connection with nature -Empowerment of the producer -Rules of use and application -Increased economic income -Higher yields -Valorization and recovery of knowledge. -Empowerment of the producer -Rules of use and application -Higher economic income -Higher yields -Valuation and recovery of knowledge.	-Impatience with fermentation time -Inadequate follow-up, dissemination, training -Lack of environmental awareness -Resistance to change -Belief that biol does not work, that sugar attracts weevils -Laziness, Rigid idiosyncrasy -Custom and ease of use of agrochemicals -Limited resources (manure, money) -Lack of time -Consumer does not value agroecological products -Competition exerted by the agrochemical industry -No habit of preparing own bioinputs -Inadequate application.
PERÚ	-Good health, quality of life, environmental care -Better yields, economic income -Dialogue of knowledge -Organizations that promote the practice -More awareness of environmental care -More demand for agroecological products -Better production, awareness of the effects of agrochemical use, soil nutrition -Close relationship with promoters -Recovery of ancestral knowledge.	-Belief that organic production is more expensive -Dead soil and constant presence of pests -Competition of the agrochemical industry -Lack of knowledge of the processes -Consumer does not value agroecology – Barely access to manure -Rigid idiosyncrasy, Lack of support from authorities -Custom to agrochemicals and resistance to change -Impatience -Sexism -Difficult access to remote communities -Analphabetism -Double discourse (organic production for self-consumption, agrochemicals for sale) -Easy to buy agrochemicals, absence of government promotion of this practice.

Reservations about biol use are also fueled by numerous considerations. Perhaps most notable in studies summaried above biol may not improve crop performance, especially if used infrequently or without usingin concert with other fertility management strategies. Growers may also have negative ideas about handling manure and concerns about organic certification if fecal coliform bacterias contaminate crops through biol. The social, political and economic context in each region also influences biol use. The cost of material inputs, such as the plastic container or availability of manure can be prohibitive. In Azuay, Ecuador the influence of agroecological movements contributes to the internalized value of sustainable agriculture and practices like biol use. Alternately, in Oruro, Bolivia quinoa production is dominated by agro-exports, and use of bio-inputs is conditioned by the requirements of the foreign market.

11. Final considerations and next steps

A review of liquid ferments applied by small holder farmers in the Andes reflects ample evidence for positive benefits to plants and soil. However despite some tradition of fermentation and enthusiasm for using slurry digest from biogas as an agroecological complement to organic nutrient inputs, both available literature and field interviews show high variability and uncertainty as to the properties of these inputs, details of their production and their utility in agroecosystems. Further assessments of the the utility of biol in smallholder systems in the Andes should address basic questions related to ferments themselves and how they are integrated into agroecological systems.

Table 14 proposes an approach for future evaluations and studies. Liquid starter ingredients are critical to the fermentation process and the agronomic utility of the biol. Any evaluation of these starting materials must equally consider the broader agroecological context, such as whether available manure is adequately utilized for the biol, and what additional nutrient management options may complement the use of the biol. Process (i.e., pH) monitoring during on-farm biol preparation will help reduce variability in assessing the agronomic value of biol, as will more careful approaches to how biol and biosol, which have different agronomic value, are harvested and applied. Finally, the properties of biol-especially those linked to material inputs-need to be evaluated in more detail, both in terms of potential risks, such as human pathogens, and benefits to plant and soil health. Careful integration of the basic issues linking biol use and agroecosystems will help address the high variability of agronomic results and the uncertainties expressed by practitioners and users. Additional information gaps are detailed below.

Table 14. Summary of considerations and recommendations at the material input, process and product evaluation levels for the use of liquid ferments (left column) within agroecological systems (right column).

	Farm-made liquid ferment	Agroecological system
Material	Tool to assess nutrient value of inputs. For example: manure + plant biomass + ash=total N, P, C. To estimates inputs to agroecosystems and compare between biol formulations.	Assess on-farm resources for biol preparation. Is diverting manure to biol sensible for soil nutrient management for a given farm? Do required materials (recipient, ingredients) make economic sense?
Process	Basic monitoring of temperature, pH, conductivity, or even odor and gas emissions to assess fermentation progress and properties.	Liquid biol, fine particles, and biosol have different nutrient properties. How they are separated and applied should be assessed in the context of local soil and crop.
Product	Measurement of nutrient inputs and outputs in biol (from above). Monitoring of pathogens for reducing risks.	Assess soil and plant health. Whats is the role of biol + biosol in broader agroecological system (i.e. compost and crop rotation).

A. Information gaps and priorities for research

Inputs and monitoring - The diversity of inputs used in liquid ferments creates opportunities to adapt ingredients to local regions, but a challenge to standardize ferments. A tool to calculate the C:N and nutrient value of inputs to estimate the final properties in biol after fermentation is one approach to address this issue (Table 14). Once the properties of inputs are estimated, monitoring parameters such as pH and conductivity will yield information that can be compared across liquid ferments and used to inform better practices. These steps amount to standardizing calculations of inputs (in terms of nutrients) and measures of the status of the fermentations over time. Ultimately this information will inform agronomic use such as application rates and adaptability to local soils and cultivars.

Inoculums - A large number of commercial inoculums and liquid ferments are marketed and sold. In some cases, these products undergo careful agronomic testing, such as the commercial bioinputs produced by PROINPA in Bolivia. However, evidence of the benefits of commercial inoculants on plants is often insufficient (Owen et al. 2015). Unsubstantiated claims run the risk of propagating myths about the contributions of inoculants and may distort real benefits worthy of future studies.

Economic analysis - Several academic theses in our review included basic information on the cost of inputs for liquid ferments compared to commercial fertilizer - suggesting a benefit for using biol for returns from crop production. Numerous additional costs are often not considered, such as cost and accessibility of large plastic fermentation containers in the rural Andes, and especially the labor of preparing ferments and applying repeatedly. Promotors and farmers in the field also routinely cited economic benefits of using biol, specifically of the prospect for premium prices for produce made with agroecological practices. A broader, critical economic analysis of household income impacts from use of farm-made liquid ferments is needed.

Human health - Handling manure and manure ferments – including composted manure and slurry from biogas plants - carries some risk of contaminating water and food with pathogens. Research indicates farm-made liquid ferments can reduce pathogen loads, though this should include studies that align with standards and monitoring defined by the WHO and other agencies – both for household safety concerns and the ability to market crops using safe agroecological practices. Some health concerns also exist for acute exposure to NH_3 vapor from biol, and accumulation of toxic levels of Zn, Cu and Mn with repeated use of digests at high input rates on the same soils (Nkoa 2014).

Agroecosystem integration - This review suggests that biol performs best in conjunction with practices such as compost amendments. For example, biol have highly available plant nutrients for rapid plant uptake or potential recovery from abiotic stress, whereas many soils and composts are slower to mineralize nutrients. Likewise, the benefits of liquid biol and solid biosol must be carefully evaluated for their distinct effects on crop performance and soil health. Future research should account for how biol fits into broader approaches to agroecosystem management, resource use and sustainable livelihoods. For example, manure and plant residues use is critical to soil fertility management on many small farms, and diverting these resources to produce biol may not be beneficial for some producers. Biol should be developed in the context of sustaining agrobiodiversity in communities of the Andes, including growing plants meant to improve growing conditions on the fields and to sustain family health (Arias Andramunio 2017).

B. Conclusions and recommendations

The use of liquid ferments on crops in the Andes is mainly due to the development of domestic biodigesters and the influence of the agroecological movement that promotes innovative plant and soil fertility management practices. The effluent from manure-based biodigesters is called biol when applied to crops. Much research has been conducted on the process of anaerobic digestion and the application of the digested material to soil and plants. This information is relevant to on-farm manufactured biol, although the final properties may be different from biogas biol.

The main products of liquid manure-based ferments are plant macro- and micronutrients released during fermentation. Diverse organic compounds, such as plant hormones and secondary metabolites from plants and microbes, also occur in biol but are less understood and more difficult to assess in terms of their impact on plants. Our review suggests the nutrients released in liquid ferments depend on starting inputs (e.g. the N and P content of the manure source) but also on the completeness of the fermentation and whether nutrients are in liquid or solid form after fermentation.

The properties of the starting ingredients also influence the fermentation process, especially values such as C:N and alkalinity. Stable temperatures and reducing the size of the starting material, such plant residues, can accelerate fermentation. Ingredients added to artisanal biol, such as vegetable or dairy products, can add plant nutrients. Sugars, such as molasses, are also commonly added and appear to stimulate microbial growth and contribute to acidity (lower pH), but there is no evidence that this facilitates the release of nutrients from primary inputs, such as manure.

Microbial inoculants, such as yeasts or lactic acid bacteria, may contribute to the fermentation of carbon sources such as added sugars. No evidence has been found that soil or EM inoculants cause the growth and abundance of microorganisms beneficial to plants. Liquid ferment conditions favor microbes that thrive under conditions of high nutrient availability and energy from carbon, and research shows that these microorganisms do not persist in the soil after application of the liquid ferment.

The pH of the fermentation and of the final product have a strong effect on the chemical forms of plant nutrients in the biol. At higher pH (i.e. neutral and above), more nitrogen can be lost as NH_3 vapor. Longer storage and increased handling releases more NH_3 over time. At acidic pH, more nitrogen is retained in the biol and more P is retained in soluble form.

Even with optimal fermentation, many solids remain in the liquid manure ferments, such as fine particles of organic matter and sediments. These solids have a high concentration of plant nutrients. Undigested fine organic matter, if mixed and suspended, contains a high nutrient content. The settled solids (biosol) are high in organic matter and P.

Evidence suggests that the use of biol is beneficial to crops, mainly due to the plant-derived nutrients that are added as foliar fertilizer, which can help crops recover from biotic and abiotic stresses. The use of biol on specific crop types and soil types should be evaluated to optimize its benefit for a given context, especially how it can best complement other strategies for ecological nutrient management in smallholder settings such as manure, compost, and legume crops. Application of biol to soil can alter soil ecosystem function, including increasing N mineralization and soil respiration. Monitoring soil health metrics can help evaluate key soil ecosystem effects from application of bio-ferments, whether direct or indirect.

Limited research exists on the use of biol by smallholder farmers. The multiple steps and relative complexity of preparation and application seem to restrict, to some extent, the use of these liquid ferments to those with a greater degree of training or experience with trial and error to maximize crop benefits. Collective preparation techniques and training processes using participatory methods can partially address this limitation. The optimization of the use of artisanal biol in agroecosystems may be different from that of biol generated by a biodigester.

Liquid ferments have the potential to carry human pathogens, such as fecal coliforms. Contamination control begins by minimizing the presence of harmful microorganisms in manure. There is some evidence that fermentation, especially for longer periods and at higher temperatures, can reduce the number of pathogens. The risk of contaminating crops with pathogenic or harmful microbes should be considered for household health, as well as for marketing and crop certification.

Several additional factors are also needed to obtain the maximum benefits from the use of biol. These include 1) demystification of its properties, potentially exacerbated by advertising of unproven commercial biofertilizers, 2) a more complete economic or life-cycle analysis of the required material and agronomic benefits, and 3) an assessment of the benefits or risks to household health and nutrition.

After an extensive literature search, we found a great need for basic research on the preparation, properties and use of liquid ferments. Here we list several priorities for advancing the research and application of artisanal bioferments:

- ❖ Develop a user-friendly tool to calculate the combined nutrient contents of the biol and its estimated yield in the crops in which it is applied (perhaps in a simple digital format). This would help standardize the preparation of liquid ferments with various ingredients readily available in each local context. Such a tool would need to pay attention to the fractionation of nutrients into the biol (liquid+suspension) and biosol (settled solid) portions of a finished ferment.

- ❖ Some simple measures to optimize the fermentation process include: reducing the surface area (i.e., chopping) of inputs such as plants, maintaining fermentation at moderate temperatures without extremes, and prolonging fermentation times when the ambient temperature is lower.

- ❖ Basic monitoring of fermentation progress should include measurement of pH and, especially, electrical conductivity (EC). These parameters are much easier and cheaper to measure than nutrient concentrations. There is no such thing as an optimal pH or EC; rather, the key is to monitor over the fermentation time to assess when each measurement stabilizes, indicating that fermentation is slowing down. These parameters can be correlated with simple proxies, such as visible gas emissions, odor or color, estimated at the farm level.

- ❖ The effect of adding different rates of carbohydrates, such as molasses, on nutrient release from manure should be studied. This could include measuring the effect of artesian biol on plant growth-perhaps the addition of carbohydrate is more important in changing the pH of the biol or other properties than in accelerating nutrient release from manure, depending on crop response.

- ❖ High levels of plant nutrients exist in the solids and small particles suspended in the biol. Simple experiments should be conducted to estimate the effect of coarse or fine filtering of the biosol, or resuspension of fine sediments to be present in the biosol, followed by controlled application to crops to estimate nutrient response. The biosol contains high concentrations of nutrients and stable carbon fractions, and should be considered a soil amendment, particularly for increasing soil organic matter and P.

- ❖ Evidence suggests that manure digests such as biol should be further tested for impacts on plant disease, whether on plant surfaces or in soil. Secondary compounds found in biol may interfere with pathogen viability, and in some cases compounds from plants with anti-disease properties, added to biol may persist after fermentation. High numbers of microbes in digests may also serve to disrupt or displace pathogens on leaf surfaces or in soil. Finally, diseases associated with plant nutrient deficiencies may be aided by biol addition.

- Other organic compounds, such plant hormones and microbial signaling compounds, may have significant impacts on plant growth promotion, though this is difficult to assess separate from the impacts of plant nutrients in the biol. The presence and concentration of these compounds will be highly dependent on materials added to the biol (plant residues, organic wastes, and even diet of animals contributing manure), the chemical properties of the biol, such as pH, and the microbes present. Simple experiments may compare biol addition with equivalent additions of basic plant nutrients to asses additive benefits of secondary compounds. Alternately microbes in biol could be separated from diluted nutrients and tested for plant growth promotion properties. At a more sophisticated level secondary compounds could be screened before and after fermentation and microbes identified and enumerated with molecular techniques.

- The above recommendations focus on optimal preparation, processing and standardization of liquid ferments. Along with this optimization, basic agronomic studies are necessary. These include: determining the frequency and rate of biol application on plant traits for different crops and monitoring soil health (chemical, biological and physical) with biol application.


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