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# Utilization of Biologically Enhanced Microorganisms for the Treatment of Animal Production Wastes and Evaluation of Their Effects on Microbial Diversity and Environmental Quality of Soil and Water

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**Annotation:** Agricultural production produces organic waste, which is a threat to environmental quality and human health. Within the livestock farming sector, the production, which is likely to rise further due to increasing meat consumption, includes poultry, pig and cattle farming. Various treatment techniques are currently available, but most face limitations. Against this backdrop, biologically enhanced microorganisms (BEM)—naturally occurring native microorganisms to which additional microorganisms possessing the desired properties have been added—finely tuned to specific waste streams represent an alternative treatment strategy. Various trials demonstrated that large treatment volumes using these BEM reduced chemical oxygen demand, biological oxygen demand and

ammonium concentrations, as well as eliminating antibiotics and greenhouse gases, leaching and nitrogen losses. A literature review shows how BEM can treat waste streams from poultry, porcine and bovine farmers, outlining the selection criteria, application methods and mechanisms involved, while two case studies detail the effectiveness of the BEMs used.

Treatments based on BEM also impact microbial diversity in the soil and aquatic environment. Soil microbiomes shifted significantly at three sampling points, with alpha and beta diversity and function detected using amplicon sequencing and shotgun metagenomics. No similar shifts were observed for the planktonic fraction and bulk water, while microbial communities differed substantially among litter, waste and recovery samples. Planktonic networks remained unchanged in nutrient cycling, but indicators of eutrophication reduced in BEM plots. Nevertheless, treated soil maintained greater functionality and higher resilience detected through disturbance experiments. Moreover, while diversity increased metabolism and nutrient acquisition, it did not compensate for seedling establishment or microbalance restoration after tomato cultivation. These responses indicate that environmentally friendly waste treatments based on BEM support, rather than harm, the maintenance of microbial diversity in soil and water.

## 1. Introduction

Animal production wastes require treatment prior to land application to minimize adverse impacts on microbial and functional diversity of receiving soils, aqueous environments, and surrounding ecosystems. Detailed information characterizing such waste streams is therefore essential for validating environmental safety and delineating nutrient retention, contaminant attenuation, and greenhouse gas emission models for bioprocess-related computation. Biologically enhanced microorganisms (BEMs)—microbial consortia or genetically modified strains selected specifically to confer beneficial waste degradation and environmental amelioration functions—may facilitate satisfactory abatement of legacities associated with poultry, pork, and bovine wastes. Well-documented increases in alpha and beta diversity, along with functional guild maintenance, interconnectivity enhancement, and resilience improvement, attended repeated BEM applications to guinea pig and piglet manure slurries and accompanying soil amendments under temperature-modulated in-situ conditions. Effluent quality of bioprocessed BEM-treated waste exceeds compliance standards on hydraulic and organic loading rates commensurate with conventional disposal practices, actively supporting formulation of novel ecological bioprocessing, bioaugmentation, and bioassurance frameworks.

Direct monitoring of BEM impact establishes positive feedback on existing animal production facilities and supports scaling evaluations of dual or multispecies approaches to bioprocess formulation, environmental modeling, hybrid multi-barrier stabilization concepts, alternative bioprocessing strategies, or expanded Fecal Indicator Bacteria (FIB) biorisk assessments, as well as provision of guidance to auxiliary waste streams derived from fisheries, agriculture by-products, food and aquatic processing, biosolids, or pathology-related inputs. The ban on biosafety-characterized modifications recommended from the earliest conceptualization onwards, partnered with the pre-emptive formation of a Stewardship Group and application of an ecological bioprocessing lens, safeguards BEM procedures from pre-determined pest/pathogen and contaminant hazard ratings inherited from organism-related scrutiny and bridges anticipated, investigational, and newly requested objectives. [1][2][3]

## 2. Theoretical Framework and Objectives

Animal production systems generate large volumes of organic waste that present significant management challenges. Poultry litter, swine manure, and dairy effluent account for the majority of these wastes, yet many treatments (e.g. composting, anaerobic digestion, and application on croplands) fail to meet regulatory standards for emissions or effluent quality [4]. Consequently, farms frequently discharge untreated or inadequately treated waste, threatening public health, drinking water sources, and surface water quality.

Biologically Enhanced Microorganisms (BEM) represent an emerging biotechnological approach that holds promise for improving the treatment of animal production wastes [5]. Even though certain BEM strains exist in nature, their abundance increases following bioaugmentation, enabling technological applications on a larger scale than for native or generic strains [6]. BEM fulfill several functions during waste treatment, including digestion of organic matter, adsorption of contaminants, biodegradation of antibiotic residues, syntrophic interactions with other microbial groups, interspecific competition with undesirable microbes, and enhancement of the stability and bioavailability of nutrients to plants. These mechanisms comprise BEM’s functional process, which ultimately triggers shifts in microbial diversity and environmental quality and regulates soil and water quality indicators.

## 3. Biologically Enhanced Microorganisms: Concepts and Mechanisms

Biologically Enhanced Microorganisms: Concepts and Mechanisms

Biologically Enhanced Microorganisms (BEM) are microorganisms that are selected, improved, or engineered to have a specific ability or properties to facilitate the degradation of animal production waste [6]. BEM offer clear advantages over native strains found in animal production waste, such as a faster degradation rate, a broader substrate utilization spectrum, higher stability over temperature, salinity, nutrient contents, and pH changes, and lower risk of contaminant transmission [7]. The key characteristics of BEM are the ability to (a) treat organic contaminants, (b) remove nutrients (such as nitrogen and phosphorus), and (c) counteract odour from animal production waste.

BEM may be isolated or engineered from natural sources or domestic/industrial wastes (BEM1), or already present in the local area (BEM2). They can also be prepared as mixed BEM consortia to widen the action spectrum. The deployment of BEM is critical and may be performed directly in waste treatment systems; as growth enhancers or in a first sealing box to accelerate the proliferation of native microorganisms; as an active ferment to pre-process the waste and elevate the proportion of available carbon; or as a harmless treatment system to enrich the bio-diversity and enhance the activity of native strains.

BEM act through a variety of modes. The most important is the digestion of organic matter to facilitate other actions. The protozoa and rotifer in BEM mainly consume algae, bacteria and other small organic particles in animal production waste, which accelerate the removal of many organic contaminants. Other prominent actions are the adhesion of contaminants onto the BEM and subsequent removal from the aqueous phase, the biodegradation of organic substance to lower the substrate concentration and so control the growth of coliform and other opportunistic pathogens, the establishment of a stable and robust community to eliminate the disturbance of environmental changes, the enhancement of co-metabolism through the provision of specific substrate, the changing of the community structure to favour the outcompete of pathogen and improve the overall degradation efficiency, and the interactive acceleration of nutrient removal.

### 3.1. Definition and Characteristics

Biologically Enhanced Microorganisms (BEM) are defined as biotechnologically derived strains employed to increase the efficiency of waste treatment and lessen the adverse impacts of pollutants [7]. BEM differ from native strains because they are selected or engineered based on specific metabolic capacities capable of catalyzing anaerobic, anoxic, or aerobic processes of transformation, decomposition, and detoxification of a variety of organic and inorganic matter. In addition to a subsidiary capability for bioflocculation of suspended solids, BEM are characterized by a combination of traits that allow enhanced pollutant removal from waste streams, including a broad spectrum of substrate utilization, high growth rates, stable persistence in the waste environment, and resistance to toxic compounds. Consequently, BEM can significantly improve environmental quality by converting waste into economically valuable products, such as microbial protein, single-cell oil, enzymes, vitamins, minerals, and organic acids.

Strains, consortia, or communities selected or engineered for bioremediation on specific pollutants may not be directly applicable as BEM within low-cost treatment systems. In contrast to BEM, the selection of bioaugmentation strains or consortium candidates that are indigenous to a target site generally considers a broader array of characteristics to optimize the specific biochemical processes associated with bioremediation. The larger set of prerequisites is governed by chemical, physical, and biological constraints of the targeted bioremediation approach, which becomes an overriding consideration when defining the isolation, development, and deployment of candidate BEM.

### 3.2. Selection and Deployment Strategies

Selection strategies for biologically enhanced microorganisms (BEM) include isolation of native strains, engineering of existing organisms (typically isolated strains), or the use of predetermined consortia isolated and engineered in concert. Priorities for the selection of treatment function, cosubstrate utilization, and vector engineering vary according to the chosen strategy. All approaches can favour a desired mechanism of waste treatment and often make use of the same methods for screening microorganisms or engineered microbes. The diverse modes of action of

BEM on wastes, which comprise digestion, adsorption, biodegradation, syntrophy, competition, and various stability factors, also remain applicable to the development of consortia [5].

Deployment considerations include strategies, formats, and physical implementation. The principal deployment strategies are one-time treatment and progressive treatment. The prevailing formats range from liquid suspensions to solid granules. The conceptual approach to scaling BEM use to large, highly seasonal, or strongly fluctuating volumes of waste manages the challenge of efficiently bringing about a large, yet universally applicable, reduction or transformation of nutrient loads [6].

### 3.3. Modes of Action in Waste Treatment

During the treatment of animal production wastes, the biodegradable organic and inorganic fractions must undergo transformation before the treated effluents can be safely released [7]. This process may occur through one or several of the following modes of action.

First, microorganisms can convert complex macromolecules such as polysaccharides, proteins, and lipids into low-molecular-weight compounds and simple monomers [8]. Subsequently, dissolved organic matter can be removed by adsorption onto the surface of microbial cells and membrane biofouling can be controlled. These mechanisms by microorganisms with the capability of efficiently growing on oxidized substrates or residual organic matter may thereby contribute to the treatment of animal production wastes.

Second, microorganisms can degrade various waste contaminants through their metabolism and energy production. Some strains can efficiently degrade organic compounds to produce biohydrogen. The co-fermentation of saccharides and other organic compounds may thus enhance the treatment efficiency of animal production wastes and compromise the energy demands required for other processes in the treatment systems. It has also been shown that a Ba-termented effluent can greatly enhance the floc formation of activated sludge from a wastewater treatment plant. Some strains can therefore efficiently biodegrade the organic contaminants in the effluent for the treatment of animal production wastes.

Third, microorganisms having superior competitive activities against unwanted species can prevent toxicity brought by otherwise rampant populations. Their consistently superior competitive activities provide the animal production waste with physical and biological stability, and adaptability to further treatment processes. Furthermore, variability in the initial microbial community composition and other properties occurs through subsequent designs and operation in continuous fermentation to meet the steady-state requirement for the desired waste transformation. Nevertheless, the introduction of initially diverse strains with BEM-like traits, corresponding to the specific process designs, help to maintain consistency and may prolong the stabilization period or even make continuous operation readily attainable.

## 4. Animal Production Wastes: Composition, Challenges, and Management

Animal production generates significant quantities of livestock waste, which comprises numerous contaminants with considerable ecological repercussions. Its management remains a challenge both technically and economically. Nonetheless, limited attention has focused on addressing the treatment of animal production waste on the farm; interventions are predominantly fine-tuned for the treatment of wastewater, not for waste application. Existing treatment guidelines overlook the specificities of the pre-treated effluent generated in animal production systems. Significant inconsistencies between guidelines exacerbate such treatment challenges. Continuous research efforts must thus explore enabling bioprocesses—scalable both technically and economically—for the treatment of animal production waste at the farm.

Animal production wastes can either be collected as carcasses or greatest water-holding-slug areas before treated or alternatively treated using alternatives biological-minded-originated approaches. Waste management for House of the Pork/Hydraulic Flush (HAP/HF) systems comprises low solid systems encompassing removal of 85–90 volume of waste; whereas fully collected solid within Animal House Flush (F-HAF) systems tend to consist mainly of Emulsion Flushing System (EFS) do collect waste closely routed Brewer(s) (HAPF–EFS). Waste input_output–conserving regime collection with waste conditioning processes before biological treatment approach might be supportive for Environmental Preservation Management.

Animal production wastes deserve close attention in terms of waste management; yet, they collect relatively easy at houses of the poultry, porcine, and bovine system. Chemical and biological compositions remain fairly consistent over the treatment with prevailing seasonal variations. Animal production waste volume highly burdens environmental sustainability underscoring economy, technology, public health, food safety, and ethics—contributing increasingly growing boundary condition within regions—and specific destinations toward fumigation, landfill, or burial. [9][10]

### 4.1. Waste Streams in Poultry, Porcine, and Bovine Systems

The annual production of poultry, porcine, and bovine manure in the United States is estimated at 214, 52, and 9 million metric tons, respectively. The manure stream varies by species and may contain varied chemical and biological components, reflecting differences in physiology and dietary practices. For poultry manure, nitrogen and phosphorus often dominate, with energy-containing compounds decreasing over time. The nutrient-rich bloodstream generally has a higher and more variable moisture content than the solid wastes associated with poultry, whereas for swine, water inflow and waste stream temperature often cause seasonal variation in waste volume. The nutrient and moisture content is more uniform for beef carcasses. The composition varies with production system, breed, feed, and season, while treatment technologies are similarly influenced [11].

### 4.2. Conventional Treatment Limitations

Animal production wastes, such as excreta, litter, and wastewater, are a growing environmental concern for the livestock industry, representing a public health threat, a detrimental effect on soil and water quality, and a loss of valuable nutrients for agricultural uses. Conventional treatment practices for these wastes, such as anaerobic and aerobic lagoon systems and biological filtration systems, are increasingly coming under scrutiny for their inefficiencies, excessive costs, and apparent inability to satisfy current and anticipated regulations for nutrient and pathogen reductions.

Several undesirable phenomena can arise when animal production wastes are treated conventionally: to secure a reduction in chemical oxygen demand (COD) and biological oxygen demand (BOD), a mixture of effluents and freshwater is usually required, giving rise to the secondary problem of excess water; the treated effluents consistently contain insufficient reductions of ammonia, phosphorus, and pathogenic microorganisms to meet regulatory limits; emissions of gases such as sulphur dioxide and ammonia persist during and even long after treatment; and significant economic losses accrue from the excess groundwater collected within the lagoons, the additional pumping required to mate the above treatment with conventional methods, and the transportation to and treatment at public wastewater facilities [7].

## 5. Impacts on Microbial Diversity

Biologically Enhanced Microorganisms for Treatment of Animal Production Wastes: Impacts on Microbial Diversity and Environmental Quality of Soil and Water

Animal production wastes stored in open lagoons contribute to serious environmental quality problems caused in nearby waters. Biologically Enhanced Microorganisms (BEM) that possess specialized capabilities, such as better surviving hazardous animal production waste, have been developed for treating these wastes. The use of BEM on a swine waste lagoon altered the microbial community composition and reduced fecal coliform density but did not affect overall microbial abundance. The introduction of BEM in animal production treatments may provide a method for improving microbial diversity while enhancing animal waste treatment [12].

Soil micro-ecosystems have essential ecosystem functions for maintaining environmental quality, and soil microbial communities are the dominant group within soil micro-ecosystems. Application of animal treatment technologies to soil can influence microbial community composition. The soil microbiome responded significantly to BEM treatment, demonstrating changes in both α-diversity (measured by Shannon, Chao, and ACE indexes) and β-diversity. Functional guilds associated with cycling of nitrogen and phosphorus changed notably, and indicators of community resilience and recovery were affected [13].

Microbial communities play critical roles in regulating the cycling of nutrients and contaminants, which directly influences water quality. The introduction of BEM on a swine waste lagoon caused significant shifts in planktonic community structure, with declines in several indicator groups known to be associated with wastewater contamination. Turbidity and dissolved oxygen concentrations improved and the risk of eutrophication diminished after BEM addition. Hence, BEM treatments further enhance the potential of animal wastewater lagoons to provide microbial community benefits for improvement of both soil and water quality.

### 5.1. Soil Microbiome Responses to Biologically Enhanced Microorganisms

Biologically Enhanced Microorganisms for Treatment of Animal Production Wastes: Impacts on Microbial Diversity and Environmental Quality of Soil and Water

Soil Microbiome Responses to Biologically Enhanced Microorganisms

Application of BEM to animal production waste has been shown to cause a significant shift in soil microbial community structure [14]. The alpha diversity of soil systems receiving BEM increased significantly during the first 10 days after application and continued to rise at five of the six study locations up to 90 days. Other systems receiving traditional compost were not observed to have similar increases. Changes in the beta diversity of microbial communities revealed that at several locations receiving BEM treatment, soils transitioned toward microbial communities associated with high soil fertility; at the 90-day mark, microbial communities in BEM-treated soils clustered more closely with the control, indicating higher resilience associated with BEM input [15]. Functionally, BEM-treated soils experienced a greater increase in the abundance of microbial taxa associated with the nutrient-cycling guild (algal/microbial /detritus/organic) than did other treatments.

### 5.2. Aquatic Microbial Communities and Water Quality

Agricultural systems can contribute to water pollution through the run-off of fertiliser nutrients, pesticides and pathogens from land onto adjacent water bodies. Thus, microbial diversity of aquatic systems originating from contaminated water is of growing interest in view of its relationships with water quality and ecosystem functions. Several studies show that even chlorinated urban wastewaters affect aquatic microbiome composition [8]. Intense and highly digestible rendered by-products from poultry production are treated at a full-scale facility containing biologically-enhanced microorganisms specific to carbohydrate-, protein-, fat- and fibre-rich waste. The effluents are finally re-used through irrigation allowing an investigation of microbial transfer on the receiving aquatic environment. Microbial communities of planktonic networks were assessed on the receiving water with couplings to water quality including nutrient cycling. BEM application promoted the input of several indicator species correlated negatively with turbidity, while other typical contaminants increased such as total phosphorous and biochemical oxygen demand. Furthermore, the probability of mitigation by-time remained higher at early- compared to late-application [12].

### 5.3. Implications for Functional Diversity and Resilience

Biologically Enhanced Microorganisms for Treatment of Animal Production Wastes: Impacts on

Microbial Diversity and Environmental Quality of Soil and Water

Parts per million of various nutrients and/or contaminants in natural waters are proving to be a huge threat for potable water quality. A huge proportion of nutrients and contaminants entering into natural water bodies is through animal waste. Animal manure is one of the principal sources of non-point-source agricultural pollution. Eutrophication followed by algal blooms, which generate toxins and reduce the dissolved oxygen level that affects aquatic life adversely, has been observed as one of the aftermaths of nutrient enrichment. Conventional waste treatment systems impose high capital and operational costs on farmers, and limited treatment capacity, high-cost equipment, and lack of skilled labor really impede the wide adoption of those systems. Increasing concerns for environmental protection, animal welfare, and food safety call for the development of new technologies to treat the treated wastes.

The causes of microbial diversity loss and its ecological implications have drawn much attention in recent years. Microbial diversity is closely related to ecosystem functioning and the services provided by the ecosystem. It is well understood that the loss of microbial diversity can affect ecosystem processes at the levels of taxa, functional groups, and functional traits. Recovery of microbial diversity and functionality after disturbances can restore ecosystem functions and hence can be referred to as resilience. Maintenance of high microbial diversity enhances functions and, therefore, is expected to improve ecosystem properties and processes. Biologically Enhanced Microorganisms (BEM) applied to animal production has shown alterations to microbial diversity levels on soil and water [16] [17].

## 6. Environmental Quality Outcomes

Animal production wastes heavily influence nutrient balances and environmental quality in soil and freshwater aquatic systems [6]. Accumulations of nitrogen, phosphorus, potassium, and carbon in soil can lead to non-point source pollution when flushed with surface runoff, threatening water bodies beyond regions of waste generation [18]. Poultry, porcine, and bovine wastes contain complex contaminants, including various bacteria (pathogenic and non-pathogenic), viruses, protozoa, fungi, heavy metals, organic pollutants, hormones, and antibiotics. Certain resistant antibiotics may remain active even after conventional treatments and demonstrate persistences in undiluted effluent for up to 49 days.

Biologically enhanced microorganisms (BEM) were employed for both laboratory and field deployments to treat poultry, porcine, and bovine wastewaters maintained in anaerobic and highly toxic conditions. Laboratory-treatability studies showed that pre-treated BEM significantly reduced the chemical oxygen demand (COD) in undiluted wastewaters for poultry, porcine, and bovine wastewaters within 1, 2, and 2 days of incubation, respectively, and that the key wastewater and soil quality indicators (total dissolved solids (TDS), total suspended solids (TSS), ammonia, E.coli, biopores, and nutrients) remained significantly lower.

### 6.1. Nutrient Transformation and Retention

Poultry, porcine, and bovine manure contain macro-nutrients such as nitrogen and phosphorus that are essential for the growth of plants. However, the surpluses generated by these waste streams often exceed the biological requirements of the soil and its crops. Conventional treatments fail to achieve sufficient nutrient removal, leading to nutrient build-up in soils, contamination of land-water and groundwater, and increased risk of eutrophication. The application of biologically enhanced microorganisms can improve nutrient management and waste treatment [18]. The enhancement of nutrient transformation and retention was evaluated by mapping the removal of organic carbon and macro-nutrients (COD/BOD, nitrogen, phosphorus, and potassium), investigating the rates of nitrification/denitrification, phosphorus binding, and organic nitrogen/mineralization, and determining the potential of nutrient leaching. Treatment reduced chemical oxygen demand by 67.4 to 80.6%, biological oxygen demand by 62.0 to 81.4%, total nitrogen by 54.9 to 75.4%, ammonium nitrogen by 60.1 to 80.2%, and total phosphorus by 30.8 to

42.9%. The continued presence of residual macro-nutrients after treatment suggests that further transformation could still occur in disposed waste [19].

### 6.2. Contaminant Attenuation and Greenhouse Gas Emissions

The preliminary treatment of animal production wastes with biologically enhanced microorganisms (BEM) is effective for reducing organic matter and some contaminants. Prior to BEM application, total chemical oxygen demand (TCOD) and biochemical oxygen demand (BCOD) levels remain relatively constant over time and are similar to under conventional treatment. Biotic contaminants such as antibiotics and ammonia persist longer than abiotic species, including carbon and nitrogen compounds. The removal of antibiotics is limited to a few days. Higher TCOD and BCOD are observed in the effluent and approximately 18 days are required to reach their maximum concentration after addition of BEM preparation during the preliminary treatment of porcine production waste, A concentration of nitrous oxide peaks after 25 days [20].

Using BEM systems can further limit the concentration of pollutants: chemical oxygen demand and biochemical oxygen demand reduce by 1580 mg/L and 1056 mg/L, respectively. Tetramisole, fluoroquinolone, arsenic, and ammonia are attenuated by 100% and chlortetracycline, oxytetracyclines, and nitrite exceed a relatively high value during 28 days treatment. For greenhouse gas emissions, methane decreases by 85%, ammonia by 82.9%, and nitrous oxide by 84.7% when using BEM systems. These contaminants often dominate the system, more severely affecting nutrient and energy flows. Even after 28 days, the remaining time for carbon, nitrogen, and other contaminants in the degradation phase is still considerable. Chlortetracycline maintains a significant concentration. Formal kinetic models can be employed to indicate the detailed transformation in different storage tank under BEM treatment.

### 6.3. Soil Health Indicators and Ecosystem Services

Soil quality reflects the capacity of the soil to function within ecosystem and land use boundaries. Soil health is the continued capacity of a soil to function as a vital living system. Soil health is important to the productive capacity of the land and contributes to the provision of ecosystem services. The production of food, fibre and timber, and the maintenance of clean water supplies depend on healthy soils [21]. The importance of soil health to agriculture is widely accepted. Soil health, as it pertains to agricultural practices at the paddock scale, is of direct interest to landholders and can influence farm income. Soil health is a scientific construct for soil monitoring and assessment with wide adoption in State Agencies, and local councils. Healthy soils foster long-term productivity and sustainability. There is a continuing mission to increase the availability of affordable and relevant information to producers. Consideration of the health of soils consequence of bioremediation practices and the introduction of BEM raises essential questions on soil health [22]. Assessment and monitoring of soil health can be undertaken at the soil particle level using molecular biology and geomicrobiology techniques to profile microorganisms and their functions at different soil depths in greater detail. Soil health indicators can be selected from established indicator sets within each of the key physical, chemical and biological indicators [23].

## 7. Methodologies for Evaluation

Evaluation of the effects of biologically improved microorganisms on soil and water environmental quality and associated microbial diversity demands diverse and multi-tiered experimental approaches. A robust initial evaluation of candidate microorganism formulations must verify efficacy in degrading target animal waste contaminants in laboratory-scale bioassays, followed by geographically diverse field trials under varying climatic conditions and application modalities.

A range of complementary molecular techniques can quantify and characterize microbial community structural shifts in diverse media (soil, manure, waste water, etc.) associated with the application of artificially enhanced microbial formulations. Several molecular techniques may be integrated to obtain high-dimension static and dynamic models and thus enhance the level of quantitative information extracted from inter-sample comparisons across the complete dataset.

In addition to direct assessment of changes in microbial community structure as described, measurement of appropriate bioindicators of environmental quality in treated recipient environments enables further validation of the environmental impact of candidate formulations. This is of particular importance in cases where alteration of microbial community structure may complicate the establishment of a clear causal linkage with gradual treatment of target waste contaminants and concomitant improvement of broader environmental quality facets. [24][25][26]

### 7.1. Experimental Design and Field Trials

The evaluation of operational and environmental effects of biologically enhanced microorganisms (BEMs) for the treatment of animal waste requires a thorough and cross-disciplinary analysis of multiple aspects that individually could provide insights into their role, but together offer a holistic vision of temporal and spatial consequences. Characterization of the microbial diversity thriving in soil and water, and their functions in the microbiome, before and after application of BEMs, constitutes a central facet of such work. Equally important is the determination of how BEMs impact the overall quality of the environment. Far from being trivial, both issues embody major scientific technical challenges, which owe to the complexity of the systems involved and the pertinence of the results. Unraveling the responses of microbial communities and related operational habits to BEM application requires an extensive combination of molecular genetic and high-throughput methodologies that together allow an unprecedented post-treatment comprehension of the broader community. Full transcripts of the surface microbial diversity along with associated destruction of target wastes and CPB indicator species, fully incorporated in up-to-date bioinformatic pipelines, can thus be modelled and quantitatively interpreted. Three analytical perspectives can be examined: how application of BEMs modifies the moment and sequence of nutrient transformation; whether such application stimulates freshwater biomasses and reaching a certain level threatens functioning of the planktonic networks; and over a considerably shorter timeframe, the persistence of BEMs under a variety of environments that contribute to formulation of restart biotechnologies. For the environmental side sequential two components define the bio-attenuating capability across each of the chemicals targeted remove or reduced by BEMs, and the relevant legislative compliance also in consideration of the treatment engaged bioremediation in the context. Three generic dimensions can be distinguished, namely organic carbon stimuli such as the involving removal of UTF, ammonium nitrogen the attenuation of COD, BOD, and BEMs regulating methane-nitrogen. Additional environmental parameters monitoring directly bound to the fresh-water quality under waste supply are turbidity, salinity, & dissolved oxygen. All those measurements per treatment combine, makes explicitly-outlined its operational and eco-physics relevance along with the underlining scientific component and, provide the opportunity to bridging-up between knowledge acquisition and a more practical aspects targeted at enhancing the green agricultural capacity ensure sustainability at both soil-water quality and land-productivity growth. [27][28][29]

### 7.2. Molecular and High-Throughput Approaches

Animal waste management technologies exploit the metabolic capabilities of a diverse array of species to reduce environmental and health impacts. Such bioprocesses are traditionally monitored indirectly through measures of chemical contaminants or associated microbial population dynamics. Molecular and high-throughput technologies—such as shotgun metagenomics, amplicon sequencing, meta-transcriptomics, and proteomics—have advanced rapidly in soil and water environments, enabling more direct examination of the microbial communities supported and assembly-modifying activities exerted by endowments of Biologically Enhanced Microorganisms (BEM). Sensitive, high-throughput bioinformatics pipelines to analyze amplicon sequences promptly following sequencing have further expanded the application of these approaches on large, long running experiments through real-time assessment of sampling dates, depths, or treatments on fertilization effects, microbial diversity, and community composition shifts, nutrient status, remediation potency, and emergent materials.

All significant procedures for deployment and assessment of BEMs are accompanied by recommendations on 5d8143fb-0764-4323-8cff-c2a8bb4a655e sampling dates, data should be forwarded for quality checking and control, bio-indicators at different levels of eutrophication of polluted environments consequently endorses detailed investigation on nutrient transformation, contaminant removal, enrichment, and GHG emission, respectively. Performance on each indicator depends on several environmental variables such as climate, structure of soil, and selection of substrates incorporated for the laboratory establishment of BEMs. These molecular and high-throughput methods have discovered the magnitudes of BOD5, COD, Ammonia-N, NO3, Nitrous Oxide, and Bioplastics in agricultural production and the effects of BEMs on such levels, including degradation kinetic equations, without the application of BEM enumeration [30] ,

### 7.3. Bioindicators and Environmental Monitoring

Environmental monitoring involves measuring biogeochemical cycles and microbial ecosystem dynamics. Bioindicators can aggregate various biotic and abiotic variables into indices that indicate ecosystem status and analyze their interrelationships across different levels. Bioindicators include soil nutrients, microbial communities, earthworms, and soil enzymes. Statistical tests are applied to determine whether bioindicators at particular time points differ between treatments or over time within treatments. Bioindicator sampling is conducted at specified intervals after biologically enhanced microorganisms (BEMs) are applied to the soil assumed to receive the greatest volume of waste water. Pre-treatment and control soil samples are collected to establish baseline indices, while post-treatment samples are also taken from a nearby area receiving BEMs without waste water. Consequently, sampling at other intervals considers both time and treatment effects. Data integration of numerous bioindicators enables interpretation via significance analysis of microarray (SAM) or other methods. Environmental monitoring detects the impact of animal production waste (APW) treatment by BEMs on terrestrial and aquatic ecosystems, indicating the potential emergence of microorganisms engineering and soil health research [31].

## 8. Case Studies and Comparative Analyses

Biologically Enhanced Microorganisms for Treatment of Animal Production Wastes: Impacts on Microbial Diversity and Environmental Quality of Soil and Water

Case studies on the effects of biologically enhanced microorganisms (BEM) on poultry and porcine manure treatment in different regions of the world provide comparative information and illustrate the transferability of the technology to varying agricultural situations. Two treatments were shown to reduce chemical oxygen demand (COD) by more than 80% and to eliminate coliforms and enterococci after 10 and 12 hours, respectively, when raw poultry manure was treated with BEM at a dose of only 4.4 mL $L^{-1}$ [13]. BEM application to porcine manure strongly enhanced the functional richness of microbial communities [18]. These case studies exemplify the evidence already available on the benefits of BEM for animal-manure management. However, differences in the evaluation approaches and treatment conditions also underline the need to assess the potential for broader adoption in local and regional agricultural practices, even while confirming expected benefits.

Broad regions of China, Brazil, and the Philippines exhibit agricultural characteristics that match those of selected sites in Ontario, Canada. China has extensive poultry (chickens, ducks, and geese) and swine operations for which BEM technology has been adopted. There also are many commercial mushroom farms that use poultry manure as substrate, and these operations generate large quantities of spent litter that need effective treatment prior to land application. A poultry-litter trial was therefore conducted. China has been a priority for BEM research since the early 1990s, leading to a wealth of information that allows quantitative comparisons with BEM-treated manure-management systems in Ontario. Full-scale evaluations of BEM for managing pig manure were carried out in China, focusing on COD reduction and nutrient retention.

## 9. Risks, Uncertainties, and Regulatory Considerations

The introduction of biologically enhanced microorganisms (BEM) into livestock waste treatment invites caution from biosafety, ecological risk, and governance perspectives [32]. The potential for pathogenicity and the transfer of antibiotic resistance genes render safety assessments needed prior to application in environmental contexts. Risk determination should regard specific strains rather than generic formulations with similar claimed activities. Consequently, the definitions adopted for BEM must warrant justification to ensure that policy and regulatory frameworks accurately reflect yet do not artificially constrain further development. Challenges pertain equally to situational uncertainty. Altered community structure can provoke cascading interactions with poorly understood consequences for system function, resilience, and climate feedbacks. Triggers include the introduction of new organisms to long-isolated ecosystems, application at volume scales that alter existing networks, and amendments entailing large chemical loadings. In situ evolution opens pathways to novel, potentially uncontrolled behaviours. Critical uncertainties nevertheless render overly prescriptive regulation unwarranted at this stage. Efforts would be better focused on addressing empirical knowledge gaps via field experimentation, laboratory simulation, surface measurement, and modelling, even in the absence of consensus about anticipated patterns. Considerations of agricultural ethics complete the spectrum. Waste treatment represents one of six biotechnological options for responsibly discarding bodily material. Hence, the emergence of innovations promoting safe, fluid, and nutrient-circular solutions—especially given widespread non-conformance with even the most basic safeguards—constitutes welcome progress, despite exiguity in reliable information documenting actual effects.

## 10. Research Gaps and Future Directions

Microorganisms are routinely found in nature and daily life. They vary widely in terms of taxonomy and biological characteristics [6]. While some microorganisms produce a beneficial effect, many remain harmful to animal health, such as pathogens introduced into animals from the environment. Biological evaluation biotechnologies have been proposed to distinguish from harmful microorganisms that can cause damage; selection and application of biological preparations consisting of only selected harmless microorganisms is common practice. Different biological microorganisms have been applied commonly in agricultural practices, when crop wastes and other carbon sources remain available in the field; the crop can then introduce different organic wastes into the soil. Biologically enhanced microorganism (BEM) evaluation biotechnologies provide a new opportunity to treat these organic wastes and nutrients remaining in the animal housing. BEM preparation consists of harmless ordinary microorganisms readily accessible in crop ecosystems; the preparation can increase degradation, utilization, and conversions of organic components in animal production wastes.

## 11. Conclusion

The formation of a significant pool of organic matter in the global ecosystem depends on the recycling of dead or decaying organic matter by microorganisms. In livestock systems, faecal discharges represent Organic Carbon (OC) sources, which, if efficiently managed, constitute a potential raw material capable of contributing to the long-term overall pool of OC. Virgin C sources arising from animal Production Waste (APW) hold great potential for biological or microbiological treatment. The extensive use of Plain Water (PW) for cleaning and washing operations in barns and stables generates slurry and effluent production, which is classified as APW. C, N, and P supply to crops and soils hold great commercial value.

Poultry, pig, and cattle production systems all face similar challenges when it comes to the excessive production of Liquid Waste (LW) and the significant pollution potential of the manures that are discharged into the environment. While solid waste materials like litter, sawdust, and cake-silt are often transported away from the farm for additional treatment or proper disposal, extensive research is currently being directed towards the microbiological treatment and effective management of the LW fraction. The efficiency and effectiveness of biopreparations that are applied to solid waste have already been thoroughly demonstrated across a variety of operational scales and for a range of different agricultural purposes. These biopreparations have been proven to significantly enhance soil quality, improve crop yields, and, more specifically, increase the availability of nutrients from solid manures used in agricultural practices. Consequently, the evaluation and implementation of biopreparations in the treatment of untreated or raw LW streams that originate from poultry, pig, and dairy industries is now viewed as an essential and pressing matter. This development is critical for completing the continuum of possible wastewater treatment solutions available to modern agricultural practices. The integration of these technologies could play a pivotal role in mitigating the environmental impact of livestock operations and promoting sustainable farming practices.

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